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INCIDENCE OF METABOLIC ACIDOSIS IN TERM PRETERM AND SMALL FOR GESTATIONAL AGE INFANTS IN RELATION TO DIETARY PROTEIN INTAKE

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During recent years acid–base homeostasis in the neonatal period has been studied from several aspects (1 2 5 6 14 19 20 21 23 24 37 38) Among the different types of neonatal acidosis the purely metabolic one appearing in the second and third week of life has been thoroughly described by Kildeberg (16 17) who named it late metabolic acidosis The mechanism of this type of acidosis especially when appearing in premature babies has been further discussed by other investigators (4 6 12 22 25 30)

The present study was performed in order to investigate the frequency of metabolic acidosis in the second and third week of life 1) in relation to maturity and gestational age of the infant at birth and 2) in relation to the protein intake during the *first weeks of life*

CLINICAL MATERIAL

A total number of 516 neonates have been studied all with an uneventful neonatal period Infants with abnormal perinatology e g perinatal asphyxia or respiratory distress syndrome have thus been excluded from the investigation Furthermore some infants who acquired an infectious gastroenteritis necessitating a change of the diet during the study period were left out.

The material was grouped according to internationally accepted standards (9) in term preterm and small-for-gestational age (SGA) infants.

Group I 334 term infants with a gestational age of 259 to 293 days i e 37 to 41 weeks of gestational age

Group II 131 preterm infants with gestational age less than 259 days i e less than 36 completed weeks of gestation

Group III 51 small for gestational age (SGA) infants with birth weight below the 10th percentile when plotted on a diagram of the relationship between birth weight and gestational age in a Swedish population (8 28)

The infants in groups I and II were all within the 10th and 90th percentiles i e the birth weight of these infants were appropriate for gestational age (AGA)

The degree of maturity was estimated by assessment of external features neurological development and head circumference (10)

Each group of infants was divided into 3 subgroups (A B C) according to the protein content of the formula given to the infants being 16 g per litre (Formula A) 22 g/l (Formula B) and 38 g/l (Formula C) The percentage of calories as protein was 9% 15% and 24% respectively The major constituents of the three different formulas are presented in Table 1 The difference in the composition of the formulas are mainly a greater concentration of protein and electrolytes in formula B and especially formula C as compared with formula A The potential net base content (PNBC) of the formulas has been calculated (see Table 1) being 15 mEq/l (Formula A) 33 mEq/l (Formula B) and 50 mEq/l (Formula C) The energy content differs only slightly

PROCEDURE AND MANAGEMENT

From day 1 to 5 after delivery all neonates were fed breastmilk or formula A. From day 6 either formula A B or C was given during the entire study period lasting until day 21 after birth The diet was chosen on an alternate basis within each group of infants, i e A-B-C-A-B-C-A-B-C- and so forth The quantity of formula fed to the infants was equal in all three groups i e 150 ml per kg per day distributed over

Table 2 *Blood acid–base variables in non acidotic infants*

Mean standard deviation (S D) and range of pH Pco and base excess (BE)

	Postnatal age (days)	*N*	pH			Pco (mmHg)			BE (mEq/l)		
			Mean	S D	Range	Mean	S D	Range	Mean	S D	Range
Group IA	5	72	7 345	0 048	7 280-7 550	41 1	2 9	35 0-36 5	−3 3	2 5	−7 0–+8 0
	15		7 376	0 0?9	7 295–7 500	39 3	3 5	32 0–45 0	−2.1	3 3	−6 5–+7 0
	21		7 374	0 067	7 295–7 550	39 6	3 1	31 0–46 0	−1 9	3 3	−6 2–+6 0
Group IB	5	126	7 3?0	0 045	7,280–7 5?0	41 3	3 3	34 0–48 0	−2 8	2 4	−6 5–+8 0
	15		7 368	0 062	7 295–7 520	39 7	3 3	32 0–47 0	−2.1	2 7	−6 0–+6 5
	21		7 389	0 066	7 290–7 540	38 4	3 1	31 0–47 0	−1 4	3 4	−6 0–+6 0
Group IC	5	1.0	7 344	0 043	7 290–7 540	40 0	3 0	32 0–46 0	−3 4	2.1	−6 0–+5 0
	15		7 350	0 050	7 285 7 540	39 1	3 0	34 0–45 0	−3 6	2 6	−6 5–+1 0
	1		7 353	0 050	7 .90–7 500	40 0	2 3	33 5–45 0	−2 5	2 3	−6 0–+5 0
Group IIA	5	52	7 332	0 030	7 295–7 410	41 7	2 5	38 0–48 5	−3 6	1 8	−6 5–+1 0
	15		7 345	0 030	7 300–7 410	40 6	1 6	38 0–43 0	−3 4	1 6	−6 5–+0 5
	21		7 353	0 030	7 300–7 450	40 5	1 9	36 5–44 0	−2 8	1 4	−5 5–+1 5
Group IIB	5	37	7 330	0 0?4	7 300–7 390	42.0	1 9	38 5–45 0	−3 5	1 1	−5 5–−1 5
	15		7 3?9	0 010	7 300–7 350	41 7	1.2	39 0–44 0	−3 9	0 6	−5 5–−2 5
	21		7 340	0 0?2	7 305–7 410	40 9	1 7	38 0–44 0	−5 6	1 1	−6 0–−0 5
Group IIC	5	15	7 322	0 0 0	7 295–7 355	41 6	2 2	39 0–45 0	−4 4	0 6	−5 0–−3 5
	15		7 332	0 014	7 300–7 355	41 4	1 5	39 0–43 0	−4 0	1 0	−6 0–−2 5
	21		7 340	0 024	7 295–7 370	40 5	2.4	38 0–46 0	−3 7	1 3	−6 0–−3 0
Group IIIA	5	11	7 311	0 014	7 295–7 345	42 0	2 7	39 0–48 0	−4 9	1 1	−6 0–−2.0
	15		7 331	0 024	7 300–7.3?0	41 0	2 4	38 0–45 0	−4 0	1 5	−6 0–−2 0
	21		7 334	0 020	7 300–7 375	41 0	3 3	37 0–48 0	−3 9	1 5	−6 0–−0 5
Group IIIB	5	19	7 331	0 0?0	7 290–7 375	41 3	3 0	38 0–48 0	−4 0	1 5	−6 0–+0 5
	15		7 369	0 035	7 295–7 445	39 1	2.2	34 0–45 0	−2 4	1 3	−4 5– 0 0
	21		7 358	0 040	7 300–7 420	39 5	2 6	37 0–48 0	−2 9	2 0	−5 5–+2 0
Group IIIC	5	15	7 399	0 052	7 295–7 500	40 1	4 0	32 0–48 0	−2 9	2 2	−6 0–+2 5
	15		7 350	0 076	7 310–7 420	39 3	2 7	36 0–43 0	−3 5	1 9	−5 0–+1 0
	21		7 386	0 043	7 300–7 490	39 3	2 6	36 0–46 0	−1.5	.5	−5 0–+3 0

p-values indicate the significance of difference by comparing subgroup A with subgroups B and C respectively within each group I II and III at the same postnatal age
$-p<0\,05$ $-p<0\,01$ $-p<0\,001$

The mean pH and base excess (values on days 15 and 21) of term and preterm infants are lower on the high protein diet (Formula C) than on the low protein diet (Formula A) By contrast in SGA infants these variables are lower on the low protein diet (Formula A) than on the high protein diet (*Formula C*)

The mean values of P_{co} are in all groups slightly decreasing with increasing age there is however no difference between the groups at corresponding intervals

Infants with metabolic acidosis

The number of infants with metabolic acidosis within each group as well as their birth weight (range) and gestational age (range) are presented in Table 3 and *Fig 1*

The pH and base excess on days 5 15 and 21 of each individual infant with metabolic acidosis are diagrammatically presented in Fig 2 (term AGA infants) Fig 3 (preterm AGA infants) and Fig 4 (SGA infants)

Relation to maturity and gestational age

In the group of 334 *term (and AGA) infants* a total number of 16 infants developed metabolic acidosis in the second week of life However they showed no clinical signs of this disturbance In 14 infants the acidosis disappeared spontaneously in the third week of life

Table 1 *Composition of formulas*

Major constituents (g/litre)	A	B	C
Protein	16	22	38
Fat	35	24	30
Carbohydrate	72	86	58
Minerals	1 7	2 6	4 2
Energy (kcal per litre)	*685*	*665*	*650*
of calories as *protein*	*9*	*15*	*24*
fat	46	34	41
carbohydrate	45	51	35
of protein as casein	40	80	80
whey	60	20	20
Major components of minerals			
Sodium (mEq/l)	9	13	17
Potassium (mEq/l)	17	35	37
Chloride (mEq/l)	13	15	18
Calcium (mg/l)	350	700	1200
Phosphorus (mg/l)	300	500	950
Iron (mg/l)	14	10	10
Magnesium (mg/l)	40	55	120
PNBC (mEq/l)	15	33	50

PNBC = Potential net base content = $(Na^{+} + K^{+} + Ca^{++} + Mg^{++}) - (Cl^{-} + 1\ 8\ P)$

5–7 meals per day The daily amount of dietary protein intake thereby achieved was 2 4 g/kg/day (Formula A) 3 3 g/kg/day (Formula B) and 5 7 g/kg/day (Formula C)

Blood acid-base analysis was determined on day 5 before instituting the dietary management as described above and was repeated on day 15 and day 21 of life Whenever a *base excess value below −8 0 mEq/l* was found measurements were repeated at least twice (after 24 and 48 hours) The infant was considered to have *metabolic acidosis when these repeated measurements confirmed the initial low base excess value*

METHODS

Blood collection

Blood samples for determination of acid-base status were collected by heel puncture following arterialisation by massaging the heel region and the application of a silicone cream (Hemo Lube® sterile Dade Reagents Inc Miami Florida) thereby obtaining satisfactory bleeding from the puncture site without or with only minor digital pressure applied In most cases crying of the baby could be avoided The blood sample was collected directly from the site of the heel puncture into four heparinized capillary glass tubes with a volume of 70 to 80 μl each which were after magnetic stirring and sealing with wax placed in 4 C refrigerator The analysis was performed within 15 to 30 minutes after sampling

Acid base analysis

The determination of the acid-base status was performed with an Eschweiler pH and Blood Gas Analyzer (Eschweiler and Co Kiel W Germany) The temperature of the apparatus was regulated to 37 C by a thermostat The pH electrode was adjusted with standard buffer solutions (Precision Buffer Solutions type S1500 with pH at 37 C 6 841±0 005 and type S1510 with pH at 37 C 7 382±0 005 A S Radiometer Copenhagen) and the P_{CO} microelectrode was calibrated with known preanalysed gas mixtures before each analysis performed Actual pH and microtonometry as well as actual P_{CO} were measured in duplicate and the mean of the duplicate was used

Correction for the actual body temperature of the infant (26) was made before calculating the base excess values from the alignment nomogram of Siggaard Andersen (27) at the actual hemoglobin concentration of the infant

Statistical methods

The mean values of the acid-base variables were compared by pair analysis using the Student's *t* test to assess the significance of differences Percentage comparisons have been made by means of the χ tests The following scale of statistical differences was used

$0\ 05 < p$	not significant	n s
$0\ 01 < p \leq 0\ 05$	possibly significant	*
$0\ 001 < p \leq 0\ 01$	significant	**
$p \leq 0\ 001$	highly significant	*

RESULTS

Non acidotic infants

In Table 2 are presented the number of infants and the mean range and standard deviation (S D) of pH P_{CO} and base excess (BE) for each subgroup on the 5th 15th and 21st day of life Apart from group III the pH and base excess are increasing with increasing age in accordance with the findings by other investigators (1 6 19 25) The infants in group III B show slightly higher pH and base excess on day 15 than on day 21

During the whole neonatal period the preterm infants (groups II A B and C) have slightly lower pH and base excess than the corresponding term and most SGA infants There is however one exception SGA infants fed the low protein formula (group III A) have lower pH and base excess than the preterm infants on the same diet (group II A)

Table 2 *Blood acid–base variables in non acidotic infants*

Mean, standard deviation (S D) and range of pH P_{CO_2} and base excess (BE)

	Postnatal age (days)	N	pH Mean	pH S D	pH Range	P_{CO_2} (mmHg) Mean	P_{CO_2} S D	P_{CO_2} Range	BE (mEq/l) Mean	BE S D	BE Range
Group IA	5	72	7 345	0 048	7 280–7.550	41 1	2 9	35 0–36 5	−3 3	2 5	−7 0–+8 0
	15		7 376	0 069	7 295–7 500	39 3	3 5	32 0–48 0	−2 1	3 3	−6 5–+7 0
	21		7 374	0 067	7 295–7 550	39 6	3 1	31 0–46 0	−1 9	3 3	−6 2–+6 0
Group IB	5	126	7 350	0 045	7,280–7.520	41 3	3 3	34 0–48 0	−2 8	2.4	−6 5–+8 0
	15		7 368	0 062	7,295–7 5.0	39 7	3 5	32 0–47 0	−2.1	2 7	−6 0–+6 5
	21		7 389	0 066	7 290–7 540	38 4	3 1	31 0–47 0	−1 4	3 4	−6 0–+6 0
Group IC	5	120	7 344	0 043	7 290–7 540	40 0	3 0	32 0–46 0	−3 4	2 1	−6 0–+5 0
	15		7.330	0 050	7 285–7 540	39 1	3 0	34 0–45 0	−3 6	2 6	−6 5–+1 0
	21		7 353	0 050	7 290–7 500	40 0	2 3	33 5–45 0	−2 5	2 3	−6 0–+5 0
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	21		7 353	0 030	7 300–7 450	40 5	1 9	36 5–44 0	−2 8	1 4	−5 5–+1 5
Group IIB	5	37	7 330	0 074	7 300–7 390	47 0	1 9	38 5–45 0	−3 5	1 1	−5 5–−1 5
	15		7 379	0 010	7 300–7 350	41 7	1.2	39 0–44 0	−3 9	0 6	−5 5–−2 5
	1		7 340	0 022	7 305–7 410	40 9	1 7	38 0–44 0	−3 6	1 1	−6 0–−0 5
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	15		7 350	0 026	7 310 7 470	39 3	2 7	36 0–43 0	−3 5	1 9	−5 0–+1 0
	21		7 386	0 043	7 300 7 450	39 3	2.6	36 0–46 0	−1 5	2.5	−5 0–+3 0

p-values indicate the significance of difference by comparing subgroup A with subgroups B and C respectively within each group I II and III at the same postnatal age
* $p < 0\ 05$ ** $p < 0\ 01$ *** $p < 0\ 001$

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Protein	16	22	38
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% of calories as *protein*	*9*	*15*	*24*
fat	46	34	41
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% of protein as casein	40	80	80
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Potassium (mEq/l)	17	35	37
Chloride (mEq/l)	13	15	18
Calcium (mg/l)	350	700	1 200
Phosphorus (mg/l)	300	500	950
Iron (mg/l)	14	10	10
Magnesium (mg/l)	40	55	170
PNBC (mEq/l)	15	33	50

PNBC = Potential net base content = $(Na^{+}+K^{+}+Ca^{++}+Mg^{++})-(Cl^{-}+1.8\ P)$

5–7 meals per day. The daily amount of dietary protein intake thereby achieved was 2.4 g/kg/day (Formula A), 3.3 g/kg/day (Formula B) and 5.7 g/kg/day (Formula C).

Blood acid–base analysis was determined on day 5 before instituting the dietary management as described above, and was repeated on day 15 and day 21 of life. Whenever a *base excess value below −8.0 mEq/l* was found, measurements were repeated at least twice (after 24 and 48 hours). The infant was considered to have *metabolic acidosis when these repeated measurements confirmed the initial low base excess value*.

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The mean values of the acid-base variables were compared by pair analysis using the Student's *t* test to assess the significance of differences. Percentage comparisons have been made by means of the χ^2 tests. The following scale of statistical differences was used:

$0.05 < p$	not significant	n.s.
$0.01 < p \leq 0.05$	possibly significant	*
$0.001 < p \leq 0.01$	significant	**
$p \leq 0.001$	highly significant	***

RESULTS

Non acidotic infants

In Table 2 are presented the number of infants and the mean, range and standard deviation (S.D.) of pH, P_{CO_2} and base excess (BE) for each subgroup on the 5th, 15th and 21st day of life. Apart from group III the pH and base excess are increasing with increasing age in accordance with the findings by other investigators (1, 6, 19, 25). The infants in group III B show slightly higher pH and base excess on day 15 than on day 21.

During the whole neonatal period the preterm infants (groups II A, B and C) have slightly lower pH and base excess than the corresponding term and most SGA infants. There is however one exception: SGA infants fed the low protein formula (group III A) have lower pH and base excess than the preterm infants on the same diet (group II A).

The highest incidence is found among the preterm (and AGA) infants 20 61 in the entire group (A+B+C) As previously noted this type of acidosis does occur even among the term (and AGA) infants 4 79% in the entire group (A+B+C) The incidence of the metabolic acidosis is thus considerably higher among the preterm infants ($p < 0\ 001$)

Relation to protein intake

All infants fed the low protein diet (Formula A) with the exception of two preterm infants achieved spontaneously normal acid–base balance within the first 3 weeks of life (Figs 2 3 and 4) Among the infants fed a higher protein diet (Formula B) every term infant but only 2 of 12 preterm infants attained spontaneously

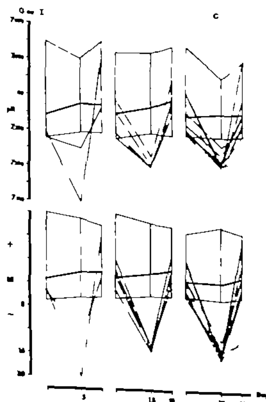


Fig 2 Group I (term (and AGA) infants) in the three dietary subgroups (A B C) pH and base excess (BE) (mEq/l) of infants developing metabolic acidosis in relation to the mean (——) and range of non acidotic infants at 5 15 and 21 days of postnatal age —— with spontaneous recovery --- without spontaneous recovery

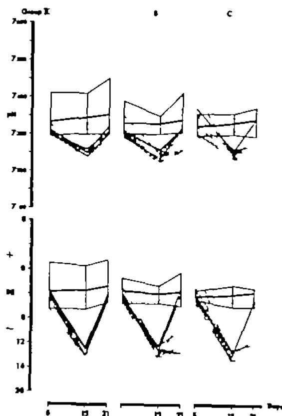


Fig 3 Group II (preterm (and AGA) infants) in the three dietary subgroups (A B C) pH and base excess (BE) (mEq/l) of infants developing metabolic acidosis in relation to the mean (——) and range of non acidotic infants at 5 15 and 21 days of postnatal age —— with spontaneous recovery --- without spontaneous recovery

normal acid–base balance Finally among those infants who were given the highest protein diet (Formula C) 8 of 10 term infants but only 1 of 9 preterm infants and none of 3 SGA infants attained spontaneously normal acid–base balance

As shown in Table 4 the incidence of metabolic acidosis in the different subgroups in comparison with group I A (term and AGA infants on a low protein diet) is increased with statistical significance only in II B and C i e preterm (and AGA) infants on the higher protein diets $p < 0\ 01$ (II B) and $p < 0\ 001$ (II C)

Among preterm infants metabolic acidosis occurs with increasing frequency with increas

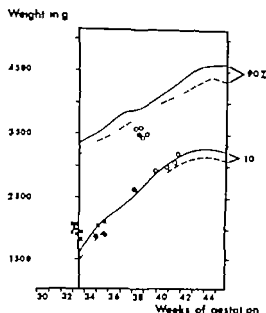


Fig 1 Birth weight by gestation of infants with metabolic acidosis plotted on a Swedish standard curve for intra uterine growth with 10th and 90th percentile for boys (——) and girls (- - -) Term (and AGA) ○ preterm (and AGA) × small for gestational age (SGA) ●

(Fig 2) The other two showed at birth signs of dysmaturity (dry cracked skin and poor subcutaneous turgor) In both of them a urinary tract infection was diagnosed

In the group of 131 *preterm (and AGA) infants* a total number of 27 infants developed metabolic acidosis in the second week of life Several of these infants showed symptoms of acidosis with signs of failure to thrive (e g poor weight gain vomiting and sucking difficulties) In 4 of 6 infants of group II A the acidosis disappeared spontaneously The gestational age was the same in all (30–33 weeks) However only 2 of 12 infants in group II B and 1 of 9 in group II C were able to correct their acidosis within the third week of life (Fig 3) It should be noted that in group II B and C the gestational age of 3 infants with spontaneous recovery was somewhat higher (35 to 36 weeks) than that of the infants who remained acidotic (30 to 33 weeks)

In the group of 51 *SGA infants* a total number of 6 infants developed a metabolic acidosis Normal acid–base balance was spontaneously attained in 2 infants whereas the metabolic acidosis was still present at 3 weeks in 4 infants (Fig 4)

It should be noted that those 6 SGA infants, who developed metabolic acidosis were not only small for gestational age (with low birth weight) but, in addition 5 of them were preterm with a gestational age less than 37 weeks This is seen in Fig 1 where the birth weight of infants with metabolic acidosis are plotted against their gestational age (8 28)

The frequency of metabolic acidosis in the second and third week of life within each group of infants is summarized in Table 4

Table 3 *Birth weight and gestational age of infants with and without metabolic acidosis in the second and third week of life*

	Infants with metabolic acidosis			Infants without metabolic acidosis			
					Birth weight g		Gestational age (weeks)
Diet	*N*	Birth weight g Range	Gestational age (weeks) Range	*N*	Mean	Range	Range
Group I							
A	2	3 400–3 550	38–39	72	3 330	2 690–4 850	37–42
B	4	2 950–3 560	37–39	126	3 428	2 850–4 200	37–41
C	10	2 560–3 490	37–42	120	3 380	2 880–4 200	37–41
Group II							
A	6	1 750–2 100	30–33	52	2 156	1 250–2 600	29–36
B	12	1 640–2 650	30–36	37	2 181	1 680–2 650	30–36
C	9	1 760–2 350	30–35	15	2 312	1 850–2 580	32–36
Group III							
A	1	1 950	37	11	2 030	1 640–2 400	37–41
B	2	1 860–1 890	34–36	19	2 159	1 790–2 550	36–42
C	3	1 300–1 895	32–35	15	2 132	1 860–2 460	35–42

Table 4 *Frequency of metabolic acidosis (MA) within each group*

p-values indicate significance of difference of frequency
N.S = not significant − 0 05 < p

	Formula A	Formula B	Formula C	Total no in each group	p-value (versus group I)
Group I (Term, AGA infants)					
Total no	74	130	130	334	
No with MA	2	4	10	16	
% with MA	2 70	3 07	7 69	4 79	—
p-value (versus group IA)	—	N S	N S		
Group II (Preterm AGA infants)					
Total no	58	49	24	131	
No with MA	6	12	9	27	
% with MA	10 34	24 49	37 50	20 61	$p < 0\ 001$
p-value (versus group IA)	N S	$0\ 001 < p < 0\ 01$	$p < 0\ 001$		
p-value (versus group IIA)	—	N S	$0\ 01 < p < 0\ 02$		
Group III (SGA infants)					
Total no	12	21	18	51	
No with MA	1	2	3	6	
% with MA	8 33	9 5_	16 66	11 76	N S
p-value (versus group IA)	N S	N S	N S		
Total no on different diets	144	,00	172	516	
No with MA	9	18	[illegible]	49	
% with MA	6 25	9 00	12 79	9 49	

−5 0 mEq/l at two measuring intervals of at least 24 hours) in the very high percentage of 41 8 of 55 premature infants On the other hand Kildeberg found in his material of premature infants (16 17) an incidence of only 8 6 However the acidosis in the first mentioned study was diagnosed at an earlier age and the degree of acidosis was not equal to that found in the material of Kildeberg or in the present study

Table 5 *Average daily change in weight in the postnatal period (day 0–21) of preterm AGA infants receiving various formulas (Group IIA B C)*

Rate of loss (−) or gain (+) in weight is expressed as grams per day (weight gain/days)
S D standard deviation
S E M standard error of the mean

Postnatal age (days)	Group IIA			Group IIB			Group IIC		
	0–5	5–15	15–21	0–5	5–15	15–21	0–5	5–15	15–21
Infants without metabolic acidosis									
N	52	52	52	37	37	37	15	15	15
Mean	−21 5	+16 5	+30 3	−24 8	+18 4	+31 5	−20 7	+19 8	+35 7
S D	9 2	5 3	9 7	10 5	8 3	7 1	8 6	6 2	7 2
S E M	3 7	2 1	4 2	3 2	2 2	2 8	2 7	1 9	3 8
Infants with metabolic acidosis									
N	6	6	6	12	12	12	9	9	9
Mean	−30 6	+12 4	+27 3	−20 0	+9 2	+17 5	−23 3	+11 4	+15 5
S D	8 2	2 7	13 5	8 2	4_	9 6	9 0	1 9	8 9
S E M	3 4	1 1	5 5	2 4	1 2	2 8	3 0	0 6	2 9
p-value of difference in loss or gain in weight	$0\ 01 < p < 0\ 05$	$0\ 001 < p < 0\ 01$	N S	N S	$p < 0\ 001$	$p < 0\ 001$	N S	$p < 0\ 001$	$p < 0\ 001$

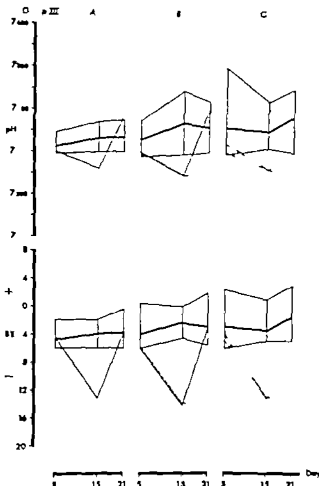


Fig 4 Group III = small for gestational age (SGA) infants in the three dietary subgroups (A B C) pH and base excess (BE) (mEq/l) of infants developing metabolic acidosis in relation to the mean (——) and range of non acidotic infants at 5 15 and 21 days of postnatal age —— with spontaneous recovery --- without spontaneous recovery

ing protein intake II A 10 34 • II B 24 49 and II C 37 50 A possibly significant in crease was found by comparing II C with II A ($p<0.02$)

Among the SGA infants an increase is also observed with increasing protein intake III A 8 33• III B 9 52% and III C 16 60 the differences are however not statistically significant The higher incidence in group III C may as mentioned above at least partly be due to the short gestational period of the infants with metabolic acidosis in this group (Table 3)

Relation to postnatal weight gain

The development of the postnatal growth in weight expressed as grams per day in the pre term AGA infants (Group II) is presented in Table 5 In the immediate postnatal period —from day 0 to 5—the mean initial decrease in body weight of acidotic infants differs slightly but without statistical significance from that of the non acidotic infants The rate of weight gain in the subsequent weeks—from day 5 to 21—is however lower in the acidotic than in the non acidotic infants of group II B as well as of group II C ($p<0.001$) The poor weight gain is probably related to the fact already mentioned that among the acidotic infants the acidosis was still present on day 21 in 10 of 12 infants of group II B and in 8 of 9 infants of group II C

In the term AGA infants (group I) and the SGA infants (group III) a tendency was similarly observed among the acidotic infants towards a slightly larger initial weight loss and a somewhat lower rate of gain in weight in the subsequent period from day 5 to 21 Since the number of acidotic infants within each sub group is relatively small in groups I and III the material does not however permit further analysis

DISCUSSION

In previous publications about late metabolic acidosis low birth weight infants who are appropriate-for gestational age have not been separated from those who are small for gestational age with the exception of the report by Glick & Allen They have in their study on metabolic acidosis (12) reported the gestational age as well as the degree of maturity of the low birth weight infants Low birth weight infants (weight at birth ≤2 500 g) usually designated as premature infants constitute in fact a heterogenous group of infants with regard to the degree of maturity which may be quite independent of the birth weight

The incidence of metabolic acidosis also varies considerably in different reports (4 6 12 15 16 22 23) Thus Ranlov & Siggaard-Andersen (22) found metabolic acidosis according to their criteria (base excess values below

Table 4. Frequency of metabolic acidosis (MA) within each group

p-values indicate significance of difference of frequency
N S = not significant = 0 05 < p

	Formula A	Formula B	Formula C	Total no in each group	p-value (versus group I)
Group I (Term AGA infants)					
Total no	74	130	130	334	
No with MA	2	4	10	16	
with MA	2 70	3 07	7 69	4 79	—
p-value (versus group IA)	—	N S	N S		
Group II (Preterm AGA infants)					
Total no	58	49	24	131	
No with MA	6	12	9	27	
with MA	10 34	24 49	37 50	20 61	p < 0 001
p-value (versus group IA)	N S	0 001 < p < 0 01	p < 0 001		
p-value (versus group IIA)	—	N S	0 01 < p < 0 02		
Group III (SGA infants)					
Total no	12	21	18	51	
No with MA	1	2	3	6	
with MA	8 33	9 52	16 66	11 76	N S
p-value (versus group IA)	N S	N S	N S		
Total no on different diets	144	00	172	516	
No with MA	9	18	22	49	
with MA	6 25	9 00	L 79	9 49	

−5 0 mEq/l at two measuring intervals of at least 24 hours) in the very high percentage of 41 8 % of 55 premature infants. On the other hand Kildeberg found in his material of premature infants (16, 17) an incidence of only 8 6. However the acidosis in the first mentioned study was diagnosed at an earlier age and the degree of acidosis was not equal to that found in the material of Kildeberg or in the present study.

Table 5. Average daily change in weight in the postnatal period (day 0–21) of preterm AGA infants receiving various formulas (Group IIA, B, C)

Rate of loss (−) or gain (+) in weight is expressed as grams per day (weight gain/days)
S D standard deviation
S E M standard error of the mean

Postnatal age (days)	Group IIA 0–5	Group IIA 5–15	Group IIA 15–21	Group IIB 0–5	Group IIB 5–15	Group IIB 15–21	Group IIC 0–5	Group IIC 5–15	Group IIC 15–21
Infants without metabolic acidosis									
N	52	57	52	37	37	37	15	15	15
Mean	−31 5	+16 5	+30 3	−24 8	+18 4	+31 5	−20 7	+19 8	+35 7
S D	9 2	5 3	9 7	10 5	8 3	7 1	8 6	6 2	7
S E M	3 7	2 1	4 2	3 2	2 2	2 8	2 7	1 9	1 8
Infants with metabolic acidosis									
N	6	6	6	12	12	12	9	9	9
Mean	−30 6	+17 4	+27 3	−28 0	+9 2	+17 5	−25 3	+11 4	+15 5
S D	8	2 7	13 5	8 2	4 2	9 6	9 0	1 9	8 9
S E M	3 4	1 1	5 5	2 4	1 2	2 8	3 0	0 6	2 9
p-value of difference in loss or gain in weight	0 01 < p < 0 05	0 001 < p < 0 01	N S	N S	p < 0 001	p < 0 001	N S	p < 0 001	p < 0 001

The occurrence in this study of metabolic acidosis in preterm (and AGA) infants on a low protein diet (10 34%) is quite similar to the frequency found by Kildeberg (8 6%) in his material of premature infants on a corresponding protein intake (17) On the other hand the high incidence in the present investigation (37 50%) among preterm (and AGA) infants on a high protein diet is comparable to the figure (41 8%) reported by Ranlov & Siggaard Andersen the protein intake in their material was about 5 g/kg/day (22)

In most infants with subsequent acidosis the initial pH and base excess values (on day 5) were found below the mean and even below one standard deviation but above the lower limit of range of the non acidotic infants in the present study Consequently infants later developing metabolic acidosis can not be selected by acid base measurements on the 5th day of life although those who have pH and base excess close to the lower limit for normal range might be considered at risk

Similarly the mean initial weight loss did not differ significantly between acidotic and non acidotic infants in this investigation and consequently the initial weight curve from day 0 to 5 after birth can not be used to predict the development of a metabolic acidosis in the second or third week of life

On the other hand after day 5 of life the rate of gain in weight was lower in the acidotic than in the non acidotic preterm AGA infants of the present material (Table 5) just as observed in previous investigations (17) The diagnostic value of this sign is however rather limited as it is found in several disorders of this age The poor weight gain is also difficult to evaluate with respect to the pathogenesis of this type of metabolic acidosis, i e whether it is an inducing factor or a result of the acidosis No conclusions regarding this problem could be obtained from the data of the present material

As shown in the present investigation the incidence of metabolic acidosis is considerably higher and the frequency of spontaneous recovery appreciably lower in preterm than in term infants In fact the majority of preterm (AGA as well as SGA) infants with metabolic acidosis did not recover spontaneously Maturity is thus an important factor for the development of metabolic acidosis in the second and third week of life

Another factor of utmost importance is the protein content of the diet it should be noted that a high protein intake in general also means a high salt intake The relation between diet and metabolic acidosis has been studied by many investigators (3 4 11 12 13 17 29 32 34 35 36) Some of them have also reported an increased incidence among premature infants fed acidified formulas (13 29 34 35 36) the conclusions are in general that acidification plays a lesser role than the protein intake for the development of metabolic acidosis (4 34 35 36)

A disorder of acid base balance provoked by a high protein intake is probably due to immaturity primarily of certain kidney functions (30 31) It should however be noted that other mechanisms for controlling total acid–base economy such as intestinal hydrogen ion regulation might also be involved (18 33)

The high frequency of metabolic acidosis among the infants receiving high protein formulas is remarkable with respect to the high potential net base content (PNBC) of these formulas (Table 1) The reason for this apparently paradoxical result is probably that—as a consequence of the rapid growth of bone and soft tissues in young infants—the PNBC of the diet is mainly retained in the body Protein catabolism with production of net acid i e sulphates phosphates and certain organic acids will on the other hand contribute to the net acid excretion in the urine and thereby to development of metabolic acidosis

Although a high protein intake has an adverse effect on the acid base balance of some preterm infants the possibility still remains that in others such an intake might under certain circumstances stimulate the rate of post

natal maturation of e g certain renal excretory functions (7 32) Those infants who in the present study developed metabolic acidosis have been studied with regard to the postnatal development of these functions The results of these investigations will later be published

SUMMARY

In this investigation comprising 516 neonates the frequency of metabolic acidosis from day 5 to day 21 of postnatal life is reported 334 term and 131 preterm appropriate for gestational age (AGA) infants and 51 small for gestational age (SGA) infants were studied The incidence of metabolic acidosis occurring after the 5th day of postnatal life was in each group of infants 4 79 20 61 and 11 76 respectively

Furthermore the infants were divided into three groups according to the amount of protein and solute content of the formula consumed An increasing incidence of metabolic acidosis with increasing dietary protein intake was observed especially among preterm infants In the group of small for gestational age infants a similar increase with a higher protein and solute content of the formula was registered However this augmented incidence was related to the gestational age rather than to birth weight

Thus the present investigation has shown that the occurrence of metabolic acidosis in the second and third week of life among low birth weight infants is primarily related to degree of maturity and length of gestation

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PLASMA INSULIN AND K VALUES DURING INTRAVENOUS GLUCOSE TOLERANCE TEST IN NEWBORN INFANTS WITH ERYTHROBLASTOSIS FOETALIS

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Newborn infants with erythroblastosis foetalis (e f) are similar in several respects to newborn infants of diabetic mothers The islet tissue in the pancreas shows hyperplasia (22 2) the insulin content in the pancreas is increased (4) hypoglycaemia is more common during the neonatal period than in normal infants (9 13) and e f infants have an elevated plasma insulin concentration at birth (1 5 23) To elucidate these factors in more detail we performed i v glucose tolerance tests and determined at the same time the plasma insulin in a group of Rhesus immunized infants

MATERIALS AND METHODS

The e.f material comprises a consecutive series of 18 infants with erythroblastosis foetalis due to Rh immunization All the infants had been admitted to the Department of Neonatal Paediatrics Rigshospitalet Copenhagen because of anti D in the maternal serum and a positive direct Coombs test in the infant. None of the mothers had a familial predisposition to diabetes mellitus none were more than 15% overweight and none had previously borne infants weighing more than 4 500 g The mean maternal age at delivery was 30 years (range 23–40) and the mean parity was 2 7 (2–4) Of the deliveries 8 were spontaneous 6 were induced by intranasally administered oxytocin and 1 by a Syntocinon® Eforam® drip whereas 3 were by Caesarean section Otherwise the deliveries were uncomplicated The e f infants clinical data are shown in Table 1 Most of them were mildly and a few moderately immunized None were hydropic and all were discharged without any signs of sequelae The birth weights was evenly distributed around the 50 percentile being in all cases within the 90 and 10 percentiles in relation to gestational age (Table 1)

As a control series we used 11 normal infants from a previous investigation (16) selecting the infants that corresponded in birth weight to the e.f infants Their mean birth weight was 3 100 g (range 2 600–3 500 g) mean gestational age 271 days (range 247 293 days) mean maternal age 21 years (16–32 years) and mean parity 1 6 (1–3) Ten deliveries had been spontaneous and one infant was delivered by Caesarean section because of placenta praevia Otherwise the deliveries as well as the neonatal periods had been uncomplicated

Three hours after birth an i v glucose load was administered through an indwelling umbilical vein catheter and blood samples were drawn through the catheter every 10 min during the subsequent 60 min Details concerning the procedure of the i v glucose tolerance test and the calculation of the *K* value have been given in a previous paper (14) Plasma glucose was determined by a glucose-oxidase method in an autoanalyzer and the coefficient of variation was 2 5% The plasma insulin concentration was measured as immunologically detectable insulin (IDI) by the method of Hales & Randle using ^{125}I insulin as tracer The plasma samples were assayed undiluted in most cases in duplicate Reproducibility of the insulin assay expressed as S E M for duplicate determinations on plasma samples were 1 3 1 3 1 5 2 6 and 6 7 µunits/ml corresponding to concentration ranges of 7 8–15 6 15 6–31 3 31 3–62 5 62 5–125 and 125–250 µunits/ml (12)

Student's *t* test with the 5% level as the limit of significance was used in the statistical evaluation

RESULTS

The mean fasting plasma insulin concentration 3 hours after birth was significantly higher in

Table 1 *Pertinent clinical data for 18 infants with erythroblastosis foetalis*

Infant no	Birth weight (g)	Gestational age (days)	Umbilical cord blood at birth		Umbilical venous plasma 3 hours after birth				No of exchange transfusions
			Hb- (g/100 ml)	Bilirubin (mg/100 ml)	Glucose (mg/100 ml)	Insulin (units /ml)	ΔI* (units /ml)	K value (/min)	
1	3 450	264	12 3	6 1	47	47	22	2 75	1
2	3 150	268	15 3	2 0	60	22	35	1 86	0
3	2 900	266	19 3	2 4	61	20	64	0 91	0
4	3 300	268	19 2	2 5	70	32	53	1 23	0
5	2 500	229	6 2	6 9	58	24	10	0 85	4
6	2 800	248	18 3	1 6	68	17	55	0 78	0
7	3 300	272	9 8	2 2	46	22	15	0 73	1
8	2 600	264	15 2	2 5	66	22	18	0 82	1
9	3 200	270	11 0	4 4	58	28	31	1 49	1
10	3 100	247	14 8	1 8	76	43	99	2 36	0
11	3 100	278	14 1	—	—	41	43	0 26	0
12	2 650	261	14 5	3 5	101	20	8	0 81	0
13	3 200	258	—	5 7	60	29	29	0 78	4
14	2 200	249	13 6	6 4	42	21	8	0 91	3
15	3 000	265	17 5	2 4	76	30	8	1 08	0
16	3 600	270	12 4	2 4	87	32	13	2 71	0
17	2 600	249	9 2	9 9	19	31	5	1 55	3
18	3 400	273	17 4	1 9	—	—	—	0 73	0
Mean	3 000	261	14 1	3 8	62	28	30	1 26	

* ΔI – Plasma insulin conc 12 min after the i v glucose load less fasting insulin conc

the e f infants than in the control group 28 and 16 μunits/ml respectively ($t = 3\ 28$ $p < 0\ 005$) and throughout the experimental period *the insulin concentration remained significantly* higher in the e f infants (Fig 1) The increase in plasma insulin concentration from 0 to 12 min after the institution of the glucose infusion (ΔI) was 30 μunits/ml in the e f infants as compared with 4 μunits/ml in the controls ($t = 3\ 53$ $p < 0\ 005$) and the K values were 1 26 and 0 68 respectively ($t = 2\ 9$ $p < 0\ 01$) The plasma insulin concentration rose evenly through the first 42 min and a maximum was attained in 42–62 min in the e f group whereas the increase in the control group was not significant (or maximal) until 52 min had elapsed

Between birth weight and fasting insulin concentrations, and between birth weight and K value significant positive correlations were found in the *e f* infants ($r = 0\ 52$ $t = 2\ 35$ $p < 0\ 05$ $r = 0\ 48$ $t = 2\ 18$ $p < 0\ 05$) whereas the correlation birth weight–ΔI was not significant Similar significant correlations have previously been found in major series of normal newborns (14 16)

In Fig 2 the e f infants are divided into *two groups* according to the course of the plasma insulin concentration during the i v GTT In one group there was a marked increase during the first 42 min and the concentration remained high during the period 42–62 min (High Insulin Response – HIR) The other group showed a moderate increase during the first 12 min and thereafter no significant changes in the plasma insulin concentration (Low Insulin Response – LIR) During the period 22–62 min after the injection of glucose the plasma insulin concentration was significantly higher in the HIR than in the LIR group The HIR group included 9 relatively *mildly immunized infants* (Nos 1 2 3 4 8 10 11 15 and 16 in Table 1) only 2 of whom were treated by exchange transfusion the mean gestational age and birth weight in this group exceeded those in the LIR group The 8 infants in the LIR group were the most severely immunized ones Of them 6 had a

Table 2 *Infants with erythroblastosis foetalis divided into two groups according to their insulin response after the i v glucose load*

HIR = High insulin response LIR = Low insulin response

Mean values of different parameters		HIR No of infants	HIR	LIR No of infants	LIR	Significance test t	Significance test p
Birth weight g		9	3 130	8	2 800		
Gestational age days		9	266	8	256		
Fasting insulin, U/ml	Plasma samples 3 hours after birth	9	32	8	24	2 22	<0 1 >0 05
K value /min	Plasma samples 3 hours after birth	9	1 56	8	0 96	2 11	<0 1 >0 05
Umb cord Hb g/100 ml	At birth	8	15 9	7	11 9	2 22	<0 1 >0 05
Umb cord bilirubin mg/100 ml	At birth	8	2 8	8	5 1	2 13	<0 1 >0 05

total of 16 exchange transfusions The *K* value and fasting insulin concentration in the plasma 3 hours after birth as well as the umbilical cord Hb concentration at birth were higher in the HIR group whereas the umbilical cord bilirubin at birth was higher in the LIR group (Table 2)

The fasting glucose concentration 3 hours after birth in the e f group was positively correlated to the umbilical cord Hb concentration ($r = 0.5$ $t = 2.01$ $0.05 < p < 0.1$) and negatively correlated to the umbilical cord bilirubin concentration at birth ($r = 0.67$ $t = 3.39$ $p < 0.005$) but there was no relationship between the fasting glucose concentration and the fasting insulin concentration ΔI or *K* value 3 hours after birth The fasting insulin concentration and the *K* value 3 hours after birth were not correlated either to the Hb or to the bilirubin concentration but ΔI was positively correlated to the umbilical cord Hb concentration and negatively to the umbilical cord bilirubin concentration ($r = 0.52$ $t = 2.28$ $p < 0.05$ $r = 0.47$ $t = 1.98$ $0.05 < p < 0.1$)

DISCUSSION

The elevated fasting insulin concentration and the elevated insulin response following glucose load in infants with e f show that these infants have hyperinsulinism at birth Since the disappearance rate of glucose—in terms of the *K* value—was significantly higher than in the corresponding normal infants the increased amount of insulin is probably biologically active

The insulin response after an i v glucose load showed the same individual marked variation that is seen in normal infants as well as in infants of diabetic mothers (10 6 16) Compared with normal infants of the same birth weight but of a higher gestational age the e f infants had a significantly higher insulin concentration before and during the i v GTT (Fig 1) On the other hand the course of the insulin concentration as well as its level corresponded to the plasma insulin concentration during i v GTT in 15 normal full term infants of an average birth weight of 4 000 g These infants had a mean value of 1 24 as compared with the e f infants 1 26 (16) In infants of insulin treated as well as of non insulin treated diabetic mothers on the other hand the maximum insulin concentration was reached as early as 10 min after an i v glucose load in order thereafter to drop evenly (16) Thus newborn infants with e f correspond in insulin response following i v GTT and in *K* value to normal infants of a higher birth weight and gestational age but differ from the infants of diabetic mothers

In previous studies normal newborn infants

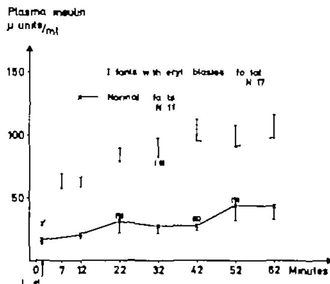


Fig 1 Plasma insulin concentration during i v glucose tolerance test in 2 groups of newborn infants

have exhibited significantly positive correlations between birth weight on the one hand and *K* value fasting plasma insulin and *ΔI* on the other (14 11 16) In the e f infants the regression lines in the named correlations were somewhat steeper and on a higher level—meaning that the fasting plasma insulin concentration and the *K* value for a given birth weight is higher—than in the above mentioned normal full term infants When considering their gestational age the e f infants were of a birth weight which did not differ from that of the normal infants Accordingly it may be assumed that the higher insulin concentration in the e f infants does not act as a growth impulse (21) perhaps because the plasma glucose concentration was not elevated at the same time The elevated *K* value corresponding to the hyperinsulinism however indicates that the extra insulin is at least biologically active with respect to the disappearance of the glucose from the blood stream

Raivio & Østerlund (23) described significant negative correlations in e f infants between fasting insulin and fasting glucose concentration and between fasting insulin and the Hb concentration measured before exchange transfusion indicating that hyperinsulinism is directly correlated to the severity of the erythroblastosis In our study there was a difference in the mean value of the investigated variables between the HIR and the LIR group (Table 2, Fig 2) These results viewed in connection with the correlations found for the entire group of e f infants between *ΔI* and the Hb-bilirubin concentration respectively actually point into the opposite direction apparently indicating a negative correlation between the insulinaemia and the severity of the erythroblastosis A theory on the cause of hyperinsulinism in e f infants based upon *in vitro* studies (24) holds that substances in the haemolysed blood of these infants are responsible for the inactivation of insulin partly by irreversible degradation caused by free SH groups in glutathione partly by reversible *binding to free haemoglobin in the plasma* In the present study however the e f infants proved to have significantly higher *ΔI* and *K* values than normal infants a finding which militates against the plasma insulin in e f infants being inactivated An increase in plasma amino acid concentration stimulates the pancreatic insulin secretion in newborn infants (7) The increased haemolysis in e f infants possibly gives a higher plasma amino acid concentration in these infants The LIR infants were the most severely affected ones and due to their lower Hb level perhaps less Hb is

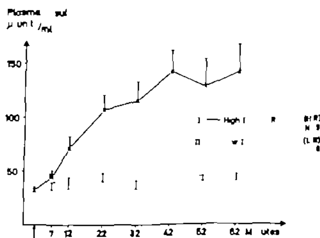


Fig 2 E f infants divided into 2 groups according to the course of the plasma insulin concentration during the i v glucose tolerance test

released Moreover organ function e g pancreatic and hepatic is more premature because of a lower gestational age

As mentioned in the introduction e f infants exhibit several functional and anatomical changes of the same nature as do newborn infants of diabetic mothers During recent years several investigations (3 18 14 15) have supported the hyperglycaemia (maternal)–hyperinsulinism (foetal) theory (19) in explanation of the changes in the infants of diabetic mothers On the other hand the above mentioned similarities between infants of diabetic mothers and e f infants have been used as arguments against this theory as the mothers of e f infants are not known to have hyperglycaemia during pregnancy

The present study has shown that the insulin response following i v glucose load is fundamentally different in e f infants and in infants of diabetic mothers and that the birth weight of the e f infants is normal in relation to their gestational age unlike that of infants of diabetic mothers who weigh an average of 550 g more than do normal infants whose gestational age is 260 days (20) Furthermore the islet hyperplasia is less marked and there are not—as in the infants of diabetic mothers—eosinophilic infiltrations around the hypertrophic islets (17)

Thus it must be considered likely that the hyperinsulinism in e f infants is of a genesis different from that in the infants of diabetic mothers and as far as the e f infants are concerned this cause is still unknown

SUMMARY

Plasma insulin was determined during i v glucose tolerance test (GTT) performed 3 hours after birth in the fasting state in 18 infants with erythroblastosis foetalis (e f) and in 11 control infants

Mean fasting plasma insulin concentration as well as the insulin concentration at each time during the i v GTT and the disappearance rate of glucose (K value) were significantly higher in the e f infants than in the control group E f infants—as a group—exhibit hyperinsulinism at birth

Between birth weight and fasting insulin concentration and between birth weight and K value significant positive correlations were found

The plasma insulin concentration after the i v glucose load showed an even increase reaching a maximum in 40–60 min in the e f group fundamentally the same pattern that was observed in the normal group but different from that in infants of diabetic mothers

It is stressed that the hyperinsulinism in e f infants is probably of a genesis different from that in infants of diabetic mothers

ACKNOWLEDGEMENTS

We wish to thank Prof. Jørgen Pedersen for his advice and Dr Jørgen Wen Foah Head of the Central Laboratory Sundby Hospital Copenhagen who kindly gave us technical assistance for the blood glucose estimations

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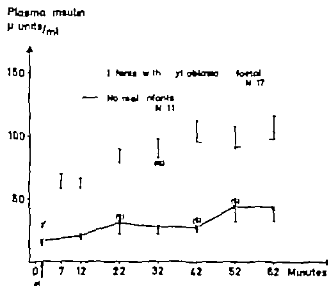


Fig 1 Plasma insulin concentration during i v glucose tolerance test in 2 groups of newborn infants

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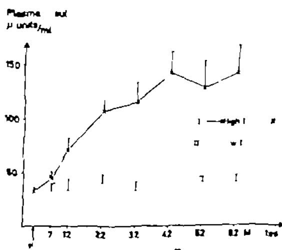


Fig 2 E f infants divided into 2 groups according to the course of the plasma insulin concentration during the i v glucose tolerance test

Acta Pædiat Scand 62 17–27 1973

VISION SCREENING OF FOUR YEAR OLD CHILDREN

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There is general agreement among ophthalmologists that discovery and correction of ocular defects early in life is of the utmost importance for the prevention of persistent amblyopia and for the achievement of an optimal binocular function (3 15 20 27 38 40) Children with large angle strabismus are easily detected usually at an early age and should of course be referred to an ophthalmologist as soon as they are observed The large groups of other eye defects however cannot be detected without a proper examination By objective methods this examination can be performed at a very early age (9 13 15) A mass screening program based on this type of examination would require a large number of professional examiners at present not available and would be very expensive (40) On the other hand if the examination is postponed to the earliest age when the cooperation of the child permits subjective tests to be used the screening procedure is highly simplified It may then be included in the organized general health service program for preschool children and performed by the ordinary staff A suitable time for testing the visual acuity seems to be the age of 4 years (1 6 8 14 18 27 30)

The purpose of the present investigation was

1 to identify children with eye disorders in an unselected population of 4-year-old children in two communities in southern Sweden

2 to make an evaluation of the findings in terms of need of professional care

3 to evaluate the screening methods

MATERIAL

Since 1967 a general health control of 4-year-old children has been organized in the city of Lund (50 000 inhabitants) and somewhat later in the community of Dalby (8 500 inhab) A vision screening program was included in this health control All children at the age of 4 years living in Lund 1967–1969 and in Dalby 1968–1969 were selected from the county population register altogether 2 573 children Out of these 2 447 children (95 1) (1 272 boys and 1 175 girls) participated in the health control

METHODS

The children were invited to participate by a letter to their parents The invitation was accompanied by questionnaires regarding *inter alia* eye disorders in the family history of birth and neonatal period and symptoms of eye disorders e g sight impairment and strabismus The ophthalmological records of children already under professional care were checked but otherwise the information from the parents was not confirmed from any records

The vision screening was performed at two Child Health Centres and took 7 min on average Besides an examination of the external eye and pupillary light reflex the following techniques were applied in the eye screening procedure

1 Examination of the monocular visual acuity with Marquez Boström s books at a distance of 5 meters as described by Nordlöw & Joachimsson (26)

2 Cover test at a distance of 0 5 m and examination of the eye movements

3 Examination of binocular vision with the Wirt Fly Stereo-test

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Key words Erythroblastosis foetalis glucose tolerance plasma insulin K value hyperinsulinism infants of diabetic mothers

willing to go through a complete eye examination on the same day were also examined by the ophthalmologist

RESULTS

Attendance and referral

Out of the 2 477 children attending the health control screening of the visual acuity could be performed in 2 397 or 98 0 cover test on 2 390 or 97 and fly test on 2 419 or 98 9 A child was considered examined if the visual acuity test plus either the cover test or the fly test could be carried out Such an examination could be performed on 2 397 children or 98 0 According to the criteria 364 children (15 2%) were referred to the ophthalmologist for further examination 349 for failing the visual acuity test 4 for failing only the cover test 8 for failing only the fly test and 3 for failing both the cover and the fly test

Six children (1 6) did not come to the examination at the Eye Clinic in spite of several reminders They are excluded from the report henceforth Thus the total number of children to calculate from was 2 391 screened and 358 referred

Clinical findings

The 358 referred children were classified according to their state of visual acuity refraction and eye muscle balance respectively (Table 1) Visual acuity of ≤ *0 6* was found in 40 8% *of them* (6 1% of all tested) and ≤0 1 in 5 9 (0 9% of all) Functional amblyopia caused by strabismus or anisometropia (4 25) was diagnosed in 12 3 (1 8 of all) Manifest strabismus was found in 10 3 (1 6 of all) The main error of refraction was hyperopia (> +2 5 D) in 28 5 (4 3% of all) Myopia was seldom found only in 3 9 (0 6% of all)

Twenty six of the 146 children with visual acuity of ≤0 6 (17 8%) and 7 of the 21 children with visual acuity of ≤0 1 (33 3%) had strabismus Out of the 37 strabismic children 26 had visual acuity of ≤0 6 (70 3%) (Fig 1)

The classification of the 358 children in

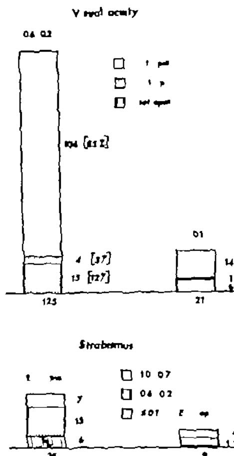


Fig 1 Correspondence between visual acuity and strabismus in 4 year-old children

terms of urgency of need of professional care is presented in Table 2

Altogether 43 / of the children (6 4 of all screened) had significant eye disorders (groups 2 and 3) and needed treatment and/or observation Correction with glasses was prescribed in 134 children (5 6) Overreferral (group 0) was found in 16 5 (2 5 of all screened)

No sex difference was found in any of the results ($p > 0.05$)

Screening tests vs clinical findings

The results of the different screening methods in relation to the ophthalmological classification are shown in Table 3 Since children born in 1963 were referred after only

Table 1 *Ophthalmological examination of 358 4 year old children referred from eye screening*

	n	% of referred (n=358)	% of screened (n=2391)
Visual acuity of poorest eye			
1 0–0 7	212	59 2	8 9
0 6–0 2	125	34 9	5 2
<0 1	21	5 9	0 9
Total	*358*	*100 0*	*15 0*
Functional amblyopia	44	12 3	1 8
Refraction			
Astigmatism >2 5	16	4 5	0 7
Hyperopia >2 5	102	28 5	4 3
Myopia >1 0	14	3 9	0 6
No or slight refractive errors	226	63 1	9 4
Total	*358*	*100 0*	*15 0*
Anisometropia >1 D	33	9 2	1 4
Position of the eyes			
Esotropia	28	7 8	1 2
Exotropia	9	2 5	0 4
Hypertropia (pure)	0	0 0	0 0
No tropia	321	89 7	13 4
Total	*358*	*100 0*	*15 0*

Items 1 and 3 were performed by specially trained nurses the other tests by a paediatrician (L K)

Criteria for referral to the ophthalmologist were (*a*) visual acuity of 5/6 or less in one or both eyes (*b*) signs of strabismus (heterotropia) (*c*) signs of defective stereoscopic vision

During the first year of screening the children who failed any of the tests were referred directly to the Eye Clinic at the University Hospital of Lund *However during the following 2 years all failures of* the vision tests were retested at a later date and referred only if failing also a second time Children under current ophthalmological care were not considered in need of further professional evaluation and were not referred

The *diagnostic investigation* of all referred children was performed by the same ophthalmologist (G S) and the same orthoptist and included the following tests and examinations

1 Visual acuity test The monocular visual acuity was checked on a projector chart at 5 m (Rodavist® illiterate E letters in a row) The row with the smallest optotypes which the child could read was recorded Five children did not cooperate well in this test and were examined with single optotypes

2 Cover test for near and for distant sight

3 Inspection of ocular movements

4 Worth's 4-dot test the classical Worth's test was used *for distant* and a modified test with pictures for near sight

5 Stereo acuity tests examined only *for near sight* with the Wirt Polaroid tests

6 Determination of the refractive state by retinoscopy 45 min after instillation of cyclopleg ic drops (*Cyclogyl® 1 *)

7 Examination of the ocular media and fundus

In most cases the 4-dioptre prism test was carried out and in the presence of amblyopia the fixation was revealed by the visuscope Only few of the children could cooperate well enough for a synoptophore examination

The results of the professional examination are expressed in a conventional way based on visual acuity *refraction and position of the eyes However we* attempted to evaluate the ophthalmological findings also in terms of need for professional care The following classification was used

Group 0 Eye examination was normal (over referred children)

Group 1 Minor eye defects were revealed but the children were not in need of treatment or repeated examination before school Examples slight refractive errors (Myopia <1 D Hyperopia 2 25–3 75 D Astigmatism < −1 5 D×0 or < −1 0 D×90) with an acuity of ≥0 6 and without significant eye muscle imbalance

Group 2 Eye defects were found considered in need of treatment (usually glasses) immediately or after re examinations Examples Myopia >1 D Hyperopia >4 D Astigmatism > −2 D×0 or > −1 5 D 90 *Anisometropia* >2 0 D Some cases of muscle imbalance without functional amblyopia were also included

Group 3 Eye defects were diagnosed where immediate professional care was considered necessary for a favourable visual prognosis Examples Manifest functional amblyopia accommodative esotropia and some other types of strabismus

In the report groups 2 and 3 are considered as *significant eye disorders*

Follow up

Children born in 1963 were again vision screened in school at the age of seven The monocular visual acuity was tested by the linear E chart at a distance of 5 m The screening was performed by the ordinary school nurses Criteria for referral to the ophthalmologist were failure to correctly reproduce 4 or more symbols on the last line (VA = ≤0 9) The professional examination was similar to that of the 4 year old children and performed by the same ophthalmologist

Control groups

44 children who failed th first vision screening test but had normal vision when re screened at the Child Health Centre were selected as a control group They were examined at the Eye Clinic by the same methods as the referrals Another 29 children who had normal findings at the first screening test and who wer

willing to go through a complete eye examination on the same day were also examined by the ophthalmologist

RESULTS

Attendance and referral

Out of the 2 477 children attending the health control screening of the visual acuity could be performed in 2 397 or 98 0 / cover test on 2 390 or 97 % and fly test on 2 419 or 98 9 A child was considered examined if the visual acuity test plus either the cover test or the fly test could be carried out Such an examination could be performed on 2 397 children or 98 0 According to the criteria 364 children (15 2) were referred to the ophthalmologist for further examination 349 for failing the visual acuity test 4 for failing only the cover test 8 for failing only the fly test and 3 for failing both the cover and the fly test

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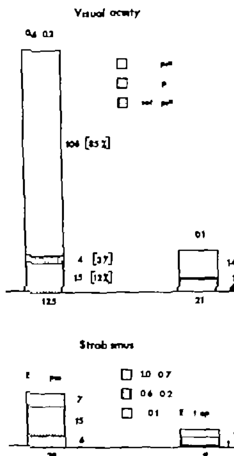


Fig 1 Correspondence between visual acuity and strabismus in 4 year-old children

Screening tests vs clinical findings

The results of the different screening methods in relation to the ophthalmological classification are shown in Table 3 Since children born in 1963 were referred after only

Table 2 *Classification of 358 4 year old children in terms of urgency for professional care*

	Ophthalmological classification				
	0	1	2	3	Total
No of children					
Boys	30	80	45	25	180
Girls	29	65	49	35	178
Total	59	145	94	60	358
% of total screened ($n=2391$)	2.5	6.1	3.9	2.5	15.0
% referrals ($n=358$)	16.5	40.5	26.2	16.8	100.0

one vision screening, they are presented in a separate column

By the visual acuity test alone 290 of the 299 children in groups 1–3 were discovered (97%) and 149 of the 154 in groups 2–3 (96.8%). When referring without re-testing (children born in 1963) 9 children (23.7%) were classified in group 1 and 15 (39.5%) in group 0 (overreferral). When referring after re-screening (children born in 1964–1965) 132 children (43.3%) were classified in group 1 and 38 (12.5%) in group 0. The difference between overreferrals among children screened once and children re-screened is highly significant ($p<0.001$).

The criterion for referral from the vision screening was a visual acuity of 5/6 or less. It was found that the lower the visual acuity screening level was, the more often was a significant eye disorder disclosed ($r=0.53$) (Table 4, Fig 2). This correlation is highly significant ($t=11.5$). It was also found that if lower criteria for referral had been used, the rate of overreferral would have diminished, but at the same time fewer children with need of

Table 3 *Ophthalmological classification in relation to screening methods*

	Opthalmological classification									
	0		1		2		3		Total	
Screening results	1963	1964–65	1963	1964–65	1963	1964–65	1963	1964–65	1963	1964–65
A										
Reduced visual acuity only	12	36	6	129	7	79	3	32	28	276
Reduced visual acuity + defective cover test	0	0	0	1	1	1	0	3	1	5
Reduced visual acuity + defective fly test	3	1	3	2	0	1	2	8	8	12
Reduced visual acuity + defective cover test + defective fly test	0	1	0	0	1	2	0	9	1	12
Total number of children with reduced visual acuity	15	38	9	132	9	83	5	52	38	305
B										
Defective cover test only	1	1	0	0	1	1	0	0	2	2
Defective fly test only	2	1	0	4	0	0	0	1	2	6
Defective cover test + defective fly test	1	0	0	0	0	0	2	0	3	0
Total number of children with defective cover and/or fly test but normal visual acuity	4	2	0	4	1	1	2	1	7	8
C										
Total number of children with defective screening tests	19	40	9	136	10	84	7	53	45	313
					154					
			299							

Table 4 *Ophthalmological classification and screening levels of visual acuity in 343 4 year old children*

Screening level of poorest eye	Ophthalmological classification				
	0	1	2	3	Total
5/6	40	97	30	8	175
5/10	13	40	44	23	120
5/15	0	4	12	10	26
<5/20	0	0	6	16	22
	53	141	92	57	343

professional care would have been disclosed (Table 4 Fig 2)

Altogether 5 children out of 154 (3 2) with significant eye disorders had normal visual acuity test but were discovered by the cover test (4 cases) or the fly test (3 cases) Thus by adding these tests another 0 2 of children with significant eye disorders were detected (Table 3)

If the cover test or the fly test had been used as the only screening methods only a minor part of the eye disorders would have been detected (Table 5)

Questionnaire

Current ophthalmological care In the questionnaires 67 children (2 8%) were reported to be under current professional care because of eye defects The ophthalmological records showed that 54 were treated for strabismus 5 for anisometropic amblyopia and 2 for high myopia Two children had congenital toxoplasmosis 1 congenital cataract 1 congenital ptosis and 2 congenital nystagmus All 67 children were judged to have significant eye disorders corresponding to our groups 2–3 These children were not considered in need of further professional evaluation and were not referred

Furthermore the records of the 126 children who did not attend the health control at all wer checked at the Eye Clinic They showed that 7 children were under current care 5 for strabismus 1 for congenital cataract and 1 for high hyperopia

Eye disorders in the families The figures of vision disturbances in the families are given in Table 6 Children with significant eye disorders discovered at the screening had strabismus or strongly impaired vision among their parents or siblings to a greater extent (13) than children with normal screening results or classified as belonging to group 1 (8) $p < 0.05$ These findings are even more evident in children already under professional care where 28 4% had parents or siblings with

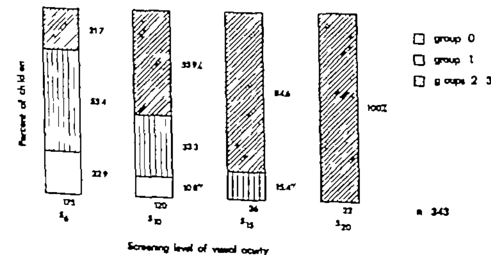


Fig Correspondence between ophthalmological classification and different screening levels of visual acuity in 343 4-year-old children

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of total screened ($n = 2\,391$)	2 5	6 1	3 9	2 5	15 0
referrals ($n = 358$)	16 5	40 5	26 2	16 8	100 0

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By the visual acuity test alone 290 of the 299 children in groups 1–3 were discovered (97%) and 149 of the 154 in groups 2–3 (96 8%) When referring without re testing (children born in 1963) 9 children (23 7%) were classified in group 1 and 15 (39 5) in group 0 (overreferral) When referring after re screening (children born in 1964–1965) 132 children (43 3%) were classified in group 1 and 38 (12 5%) in group 0 The difference between overreferrals among children screened once and children re screened is highly significant ($p < 0\ 001$)

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Reduced visual acuity + defective fly test	3	1	3	2	0	1	2	8	8	12
Reduced visual acuity + defective cover test + defective fly test	0	1	0	0	1	2	0	9	1	12
Total number of children with reduced visual acuity	15	38	9	132	9	83	5	52	38	305
B										
Defective cover test only	1	1	0	0	1	1	0	0	2	2
Defective fly test only	2	1	0	4	0	0	0	1	2	6
Defective cover test + defective fly test	1	0	0	0	0	0	2	0	3	0
Total number of children with defective cover and/or fly test but normal visual acuity	4	2	0	4	1	1	2	1	7	8
C										
Total number of children with defective screening tests	19	40	9	136	10	84	7	53	45	313
					154					
			299							

Table 4 *Ophthalmological classification and screening levels of visual acuity in 343 4 year old children*

Screening level of poorest eye	Ophthalmological classification 0	1	2	3	Total
5/6	40	97	30	8	175
5/10	13	40	44	23	120
5/15	0	4	12	10	26
<5/20	0	0	6	16	22
	53	141	92	57	343

professional care would have been disclosed (Table 4 Fig 2)

Altogether 5 children out of 154 (3 2 /) with significant eye disorders had normal visual acuity test but were discovered by the cover test (4 cases) or the fly test (3 cases) Thus by adding these tests another 0 2 of chil dren with significant eye disorders were de tected (Table 3)

If the cover test or the fly test had been used as the only screening methods only a minor part of the eye disorders would have been detected (Table 5)

Questionnaire

Current ophthalmological care In the ques tionnaires 67 children (2 8) were reported to be under current professional care because of eye defects The ophthalmological records showed that 54 were treated for strabismus 5 for anisometropic amblyopia and 2 for high myopia Two children had congenital toxo- plasmosis 1 congenital cataract 1 congenital ptosis and 2 congenital nystagmus All 67 children were judged to have significant eye disorders corresponding to our groups 2–3 These children were not considered in need of further professional evaluation and were not referred

Furthermore the records of the 126 chil den who did not attend the health control at all wer checked at the Eye Clinic They showed that 7 children were under current care 5 for strabismus 1 for congenital cataract and 1 for high hyperopia

Eye disorders in the families The figures of vision disturbances in the families are given in Table 6 Children with significant eye disorders discovered at the screening had strabismus or strongly impaired vision among their parents or siblings to a greater extent (13) than children with normal screening re sults or classified as belonging to group 1 (8 %) $p<0.05$ These findings are even more evident in children already under professional care where 28 4 had parents or siblings with

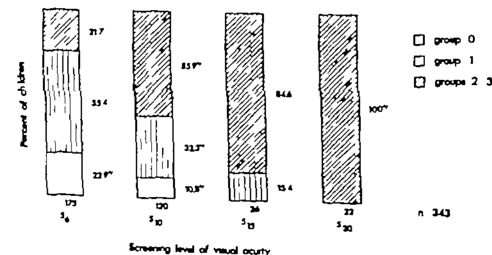


Fig 2 Correspondence between ophthalmological classification and different screening levels of visual acuity in 343 4-year-old children

Table 5 *Efficiency of screening for eye defects with cover test and fly test Result of 358 four year old children referred from vision screening*

Indication for referral	Children referred ($n=358$)		Children over referred (group 0) ($n=59$)		Children with eye disorders (groups 1–3) ($n=299$)		Children without significant eye disorders (groups 0–1) ($n=204$)		Children with significant eye disorders (groups 2–3) ($n=154$)	
	n		n		n		n		n	
Failing cover test alone	26	7 3	4	6 8	22	7 4	5	2 5	21	13 6
Failing fly test alone	44	12 3	9	15 3	35	11 7	18	8 8	26	16 9
Failing cover test and/or fly test	54	15 1	11	18 6	43	14 4	21	10 3	33	21 4

strabismus or strongly impaired vision $p<$ 0 001

Partus and neonatal period Children in group 2–3 had not a higher rate of prematurity (birth weight <2 500 g) or neonatal complications (strong icterus or cyanosis, convulsions, or breathing difficulties) than children without significant eye disorders, $p>0$ 05 (Table 6)

Somewhat more partus complications (abnormal presentation, forceps, vacuum extraction caesarean section) were reported for children in groups 2–3 15 6% vs 9 4° $p<0$ 05

In children with earlier known eye disorders there was a definitely higher frequency of prematurity than in children without significant eye disorders 11 9% vs 3 0° $p<$ 0 001

Present complaints Symptoms of disturbed vision (eye strain difficulties to see small things near or at a distance or to watch TV) were reported more often in children with newly discovered significant eye disorders (5 8%) than in healthy children (1 7%) $p<$ 0 001 and most often for children already under ophthalmological care (35 8°) (Table 6)

Table 6 *Information from the questionnaire in relation to actual findings of eye disorders*

Questionnaire	Actual findings of eye disorders: Children with significant visual defects ($n=154$)		Children already under ophthalmological care ($n=67$)		Children without significant visual defects ($n=2170$)		Total ($n=2$ 391)	
	n		n		n		n	
Strongly impaired vision or strabismus among								
Parents or siblings	20	13 0	19	28 4	174	8 0	213	8 9
Other relatives	17	11 0	9	17 4	186	8 6	212	8 9
Both parents/siblings and other relatives	5	3 3	4	6 0	47	2 2	56	2 3
Premature (<2 500 g)	8	5 2	8	11 9	66	3 0	82	3 4
Partus complications	24	15 6	7	10 5	203	9 4	234	9 8
Neonatal complications	13	8 4	7	10 5	153	7 0	173	7 2
Present complaints of disturbed vision	9	5 8	24	35 8	36	1 7	69	2 9

Control groups

Out of the 44 children who failed the visual acuity test the first time but had normal vision when re tested 31 were considered as normal by the ophthalmologist 10 were classified in group 1 and 3 in group 2 The three children in group 2 were all hyperopic (+3 5–+5 0) and were treated with glasses No case of amblyopia or strabismus was found Cover tests and fly tests were normal both at the screening examination and at the diagnostic examination

Out of the 29 children with normal visual acuity at the first screening 23 were normal (group 0) and 6 were classified in group 1

Follow up at school

In the autumn of 1970 639 children born 1963 went to school in Lund Of these 479 (74 8) were previously vision screened at the age of 4 years 139 (21 8 %) had moved into the city between 4 and 7 years of age 15 (2 3) had failed to attend the health control and 7 (1 1 %) were incompletely screened at the age of 4 years

At the visual acuity test at school 75 children failed and were referred to the Eye Clinic Half of them 37 children had earlier known eye disorders Of the remaining 38 children 25 were normal or had only slight errors of refraction without need of treatment Eleven children had reduced vision secondary to refractive errors but without functional amblyopia All were treated with glasses Seven of them had passed the screening at 4 years of age 4 did not participate The remaining 2 children had amblyopia and a steady eccentric fixation Neither of them had participated in the preschool screening

A full report from the follow up and treatment of the age classes born 1963–1965 will be given in due course

DISCUSSION

Visual disturbances in children fulfil the requirements that motivate directed screening procedures they are common (19 22 37) they may cause serious handicap later in life (37 40) simple reliable and inexpensive screening methods (22 40) and efficient treatment are available (12 20 27) Therefore it was obvious that a screening for eye disorders should be an important part of a general health control of 4 year old children To obtain the desirable high attendance at a screening program the parents must recognize the investigation as being of importance for their children s health The attendance of 95 1 % in our vision screening study was attained probably because it was included in an extensive general health control

There is an abundant literature about vision screening in pre school ages with great variations of the results Thus ratios of children referred for professional examination varies from 1 4 to 21 9 (2 5 6 10 16 18 19 22 23 27 28 32–36 42) Also the ophthalmological findings of the referred children show great disparity These divergencies are obviously due to biased samples and to differences in screening methods of criteria for referral and of professional evaluation and follow up A complete study of an unselected population of pre school children in an area has not been presented before

In the conventional division of the referred children (presented in Table 1) the limits between the different subgroups of visual acuity are of course arbitrary An isolated determination of the visual acuity however is of limited value since it reveals nothing about the underlying nature of the visual defect and does not give a reliable conception of the visual prognosis Thus the prognosis in a child with a visual acuity of 0 5 may be quite different if the cause is a simple myopia or it is a hyperopic anisometropia A strict prognostic evaluation can be made only if other aspects of the visual function also are taken into consideration Based on a complete ophthalmological examination including visual acuity objective refraction state of binocular vision and on repeated follow up examinations such a prognostic classification is suggested

The ultimate purpose of a health control of this kind is to detect disorders in children where early treatment would be advantageous This is true of several kinds of visual defects here presented as significant eye disorders (groups 2 and 3) The children in group 3 needed immediate treatment, e g glasses occlusion pleoptics or surgery It was considered that delayed treatment of these children should have seriously impaired their visual prognosis, e g by the development of a steady eccentric fixation (24)

Most of the children with reduced vision had refractive errors without signs of functional amblyopia Early optic treatment of these children may not necessarily change the visual prognosis It is however, beneficial for the children to become accustomed to glasses which will in any case be needed when school begins These constitute the major part of group 2 Other children in this group had an intermittent exotropia where orthoptic treatment or surgery should be instituted before school A few cases of esotropia without amblyopia prompted for careful observation and were also included in this group

A large proportion of the referred children (40 5°) was classified in group 1 The indication of referral was verified, but the ophthalmological examination revealed only minor refractive errors where neither treatment nor observation were indicated in the pre school years In school however when the demands of visual ability are increased some of these children may need corrective glasses

The prognosis of visual defects of children at this age may be difficult to evaluate after only one professional examination Thus, there are children with reduced visual acuity which cannot be explained by the ophthalmological examination Moreover it may be difficult to verify a functional amblyopia after one diagnostic investigation Some children have a slightly reduced visual acuity at this age but may later develop an amblyopia they may be considered as having potential amblyopia (24) By a careful follow up during 1–3 years after the first professional examination these cases could be classified with a certain degree of accuracy

A vision screening is not only an examination of the vision it is a communicative process as well (20 26 41) Therefore the normal ophthalmological findings in some of the children who failed the screening tests might well be due to their lack of cooperation or their failure to understand the instructions at the screening This fraction (group 0) decreased significantly when the children were re examined before referral (39 5 vs 12 5 •) Another small fraction (2 0 ₀) could not cooperate at all at the screening examination As a rule these children were retested when they were older but they are not included in this report

For children detected before 4 years of age large angle strabismus of congenital type was the most common reason for ophthalmological care Most of the cases with significant eye disorders detected at the age of 4 years did not squint and those who did squint (10 3 or 1 6*₀ of all screened) had mostly an accommodative esotropia which is not easily detected without a proper examination In total the prevalence of strabismus known or detected by screening of 4-year old children in this area was just below 4 As strabismus is seldom developed after the age of 4 years (17, 31) this figure is comparable to those found in examinations of older children 3 1 – 4 4• in school children (1 7 34)

Including children with known eye disorders it may be estimated that around 8 • of 4 year old children need glasses

The screening method chosen for the visual acuity test Marquez Bostrom s hooks is one of the most widely used in Sweden The test is very easy to handle and it fulfils high demands on screening accuracy (26) A still better method to detect functional amblyopia would be to use a linear E chart (4 39) which however is more time consuming and requires repeated visits to make smaller children familiar with the testing procedures (21 24) At the

diagnostic examination and at the screening at school however this more accurate linear E chart was used

In order to detect strabismic children with normal visual acuity a cover test was included in the screening methods As an amblyopic and/or strabismic child usually has an im paired state of binocular cooperation it seemed reasonable to study the efficiency of a test of the binocular function A test suitable for this age group is the Wirt Fly Stereo-test which gives a rough estimation of this visual function Tests for heterophorias were omitted since the pathological significance of hetero phorias in pre school children is difficult to evaluate

The majority of our findings of eye disorders (97) were detected by the visual acuity test The other screening methods the fly test and the cover test revealed very few additional children with significant eye disorders

Neither the cover test nor the fly test alone or together was sufficiently efficient in detect ing significant eye disorders but correspond ingly their rate of overreferral was also rather low (Table 5) Similar results have also been found by other authors (20 29 35 41) and thus both methods are too unreliable to be used as the only tests in a pre school vision screening program In an ambitious program however they may be used as a complement to the ordinary visual acuity test

There were considerable difficulties in find ing volunteers for the control examination from children who had *just passed the vision* screening test Although small these groups showed that no children with amblyopia or strabismus passed the screening tests Three children however were considered to need glasses because of hyperopia (group 2) and were thus falsely negative

A further and even stronger indication of the efficiency of the adapted screening proce dure is the results from the follow up at school Only 2 children with amblyopia were found neither of them had participated in the pre school vision screening Another 7 children out of 479 previously screened (1 5) needed glasses because of refractive errors which had not been detected at the age of 4 years Four of them however had a myopia which may have developed between the two examinations

Thus the present procedure of visual acuity testing is accurate enough as a screening method for eye disorders in pre school chil dren Considering children in group 1 as un necessary referrals however will increase the rate of overreferral from 2 5 to 8 6 i e more than half of the number referred (Ta ble 2) By lowering the passing standards of the test from 5/6 to 5/10 this referral would be reduced to 57 children or 2 4 (57/2391) (Table 4) Such a modification however could hardly be accepted since 21 7 of children needing treatment would then have remained undiscovered (Fig 2)

As could be expected (11) children with earlier known or newly discovered significant eye disorders had more often strabismus or strongly impaired vision among their parents and siblings than other children A secondary benefit from this vision screening program was that siblings of children with eye disorders were offered an ophthalmological examination and in this way several additional cases of significant eye disorders were detected

Although children reported to have family eye diseases partus complications and present complaints about the eyes seem to run a cer tain risk of having significant eye disorders this information from the parents was not *selective enough to be of practical value* as a screening instrument Therefore to detect visual disturbances in pre school children it is necessary to perform a screening examination preferably included in the general child health supervision program The visual examination should be repeated when school begins

SUMMARY

Included in a general health control of an un selected population of 2 447 four year old chil

The ultimate purpose of a health control of this kind is to detect disorders in children where early treatment would be advantageous This is true of several kinds of visual defects here presented as significant eye disorders (groups 2 and 3) The children in group 3 needed immediate treatment e g glasses, occlusion pleoptics or surgery It was considered that delayed treatment of these children should have seriously impaired their visual prognosis e g by the development of a steady eccentric fixation (24)

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The prognosis of visual defects of children at this age may be difficult to evaluate after only one professional examination Thus there are children with reduced visual acuity which cannot be explained by the ophthalmological examination Moreover it may be difficult to verify a functional amblyopia after one diagnostic investigation Some children have a slightly reduced visual acuity at this age but may later develop an amblyopia they may be considered as having potential amblyopia (24) By a careful follow-up during 1–3 years after the first professional examination these cases could be classified with a certain degree of accuracy

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For children detected before 4 years of age large angle strabismus of congenital type was the most common reason for ophthalmological care Most of the cases with significant eye disorders detected at the age of 4 years did not squint and those who did squint (10 3% or 1 6 % of all screened) had mostly an accommodative esotropia which is not easily detected without a proper examination In total the prevalence of strabismus known or detected by screening of 4 year old children in this area was just below 4 % As strabismus is seldom developed after the age of 4 years (17 31) this figure is comparable to those found in examinations of older children 3 1 %–4 4 % in school children (1 7 34)

Including children with known eye disorders it may be estimated that around 8% of 4 year-old children need glasses

The screening method chosen for the visual acuity test Marquez Bostrom's hooks is one of the most widely used in Sweden The test is very easy to handle and it fulfils high demands on screening accuracy (26) A still better method to detect functional amblyopia would be to use a linear E chart (4 39) which however is more time consuming and requires repeated visits to make smaller children familiar with the testing procedures (21 24) At the

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dren a vision screening was performed using a visual acuity test (Marquez Bostrom s hooks) cover test and Wirt Fly Stereo test The screening could be carried out in 98% of the children 364 children (15 2%) were referred because of newly detected visual defects and 358 children (15 0%) were professionally examined Of these 40 8% had a visual acuity of $< 0\ 6$ and 5 9% of $< 0\ 1$ Functional amblyopia was found in 12 3% and manifest strabismus in 10 3% The main error of refraction was hyperopia ($\geq 2\ 5$ D) diagnosed in 28 5 while myopia was infrequent 3 9%

The children examined by the ophthalmologist were also classified into four groups, according to their need of professional care where group 0 means overreferral and groups 2–3 represent significant eye disorders in need of ophthalmological treatment and/or observation Overreferral was found in 16 5% and significant eye disorders in 43% With the visual acuity test 97% of the children with eye disorders were detected Retesting children who failed the tests reduced the overreferral from 39 5% to 12 5% ($p < 0\ 001$) By lowering the passing standards of the visual acuity test still fewer children would have been overreferred but at the same time 1/5 of children needing treatment would then have remained undiscovered

Including children already under professional care the prevalence of strabismus in this unselected material of 4-year old children was just below 4% and the need for corrective glasses around 8%

Children reported to have family eye disorders, partus complications or present eye complaints were in the risk zone for suffering significant eye disorders, but this information from the parents was not sufficiently selective to be of practical value as a screening method

A small control group of 73 children and a follow-up of 479 children at school 3 years later revealed that no children with functional amblyopia were missed at the screening test

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SAPHENOUS VEIN AUTOGRAFT ARTERIOVENOUS FISTULA FOR EXTENDED HEMODIALYSIS IN CHILDREN

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The arteriovenous fistula introduced by Brescia et al (1) in 1966 to obviate the complications occurring with the Teflon Silastic (Quinton–Scribner) A V cannula (2) during extended hemodialysis has been used successfully in many adults This procedure has not been applied extensively in children because of small vessel size and poor blood flow A recent technique (3 9 10) to gain access to arterial and venous blood for repetitive hemodialysis in children has been employed during the past 2 years at Childrens Hospital of Los Angeles This report describes our experience with use of a saphenous vein autograft implanted subcutaneously in the forearm or leg for extended hemodialysis in 21 patients

MATERIAL

Twenty two saphenous vein A V fistulas were constructed in 21 patients aged 6 / to 20 / years A U shaped loop fistula was created in the forearm in 18 patients utilizing the brachial artery and cephalic a straight fistula utilizing the radial artery and cephalic vein in 2 patients and a U shaped fistula utilizing th saphenous vein and femoral artery was constructed in the anterior aspect of the thigh in one patient The age and weight of the patient at time of insertion type of fistula and complications thereof are shown in Table 1 Eleven patients (nos 1 2 4 7 8 9 12 13 14 17 and 21) had been undergoing extended hemodialysis with the use of a Teflon Silastic external shunt for periods of 2 to 24 months prior to fistula creation

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METHODS

Surgical technique

The greater saphenous vein was removed from the thigh after carefully ligating all tributaries Leaks were identified and the vein dilated by irrigating with heparinized saline The brachial artery and bracheocephalic vein were exposed through a transverse incision in the anticubital fossa of the non dominant arm A transverse parallel incision in the upper half of the forearm was used to develop superficial separate but parallel subcutaneous tunnels between the two transverse incisions The tunnels were used to accommodate the limbs of the U shaped venous loop The valves were placed in the direction of blood flow The ends of the vein were anastomosed to the sides of the brachial artery and bracheocephalic vein using fine cardiovascular sutures After flow was established the bend of the U was buried under the distal transverse incision and the incisions closed

One of us (QRS) simplified the technique by fistulizing the radial artery at the wrist to the brachial or cephalic vein in the anticubital fossa through a single subcutaneous tunnel

The technique for establishing a fistula in the thigh was similar The saphenous vein was not removed from the common femoral vein but the distal transected segment was looped through the 2 parallel subcutaneous tunnels and anastomosed to the superficial or common femoral artery

Dialysis technique

The skin over the fistula was prepared with Betadine® After infiltration of puncture sites with local an

Table 1 *Patient data*

Pat no	Age at time of insertion y	mo	Weight at time of insertion (kg)	Type of fistula	Complications
1	20	8	53 5	Loop	None
2	18	8	64 5	Loop	Scarred limbs of fistula
3	17	8	39 0	Loop	Clotted poor flow
	18	8	42 0	Loop	None
4	18		60 5	Loop	None
5	17	10	36 0	Straight	None
6	17	7	57 5	Loop	None
7	17	7	59 0	Thigh	None
8	17	5	50 5	Loop	None
9	15	10	36 0	Loop	Clotted scarred limbs of fistula
10	15	8	43 5	Loop	None
11	15	7	39 5	Loop	None
12	15		40 0	Loop	Clotted revised
13	14	2	67 5	Straight	None
14	12	11	28 0	Loop	None
15	12	9	30 5	Loop	None
16	12	1	38 0	Loop	None
17	10	3	25 0	Loop	None
18	9	11	15 0	Loop	Clotted poor flow
19	9	1	28 5	Loop	None
20	9		33 0	Loop	Clotted poor flow
21	6	9	33 0	Loop	None

esthesia (Xylocaine® 1%) venipunctures were performed with 16 gauge (Butterfly® 16 Abbott) siliconized needles in the well dilated autograft A tourniquet was applied proximally only when the fistula was insufficiently dilated The needles were taped securely in place and then connected to the appropriate arterial and venous dialyzer tubing A blood pump (Cole Parmer®) on the arterial side of the dialyzer was used to propel the blood at rates of 125 300 ml/min At the end of the dialysis the needles were withdrawn and pressure was applied manually to the venipuncture sites for 5–10 min until bleeding ceased If bleeding from the sites persisted a pressure dressing was applied for 2–4 hours The remainder of the dialysis technique was identical to that reported previously (2 4)

RESULTS

Fistula survival

Twenty two saphenous vein autograft A V fistulas were constructed in 21 patients Fistula survival length of time utilized for repetitive dialysis and present status are shown in Fig 1 Sixteen fistulas have been used for periods of 1–10 months Two patients (nos 4 and 19) have functioning fistulas which have not as yet been used Patient 14 died suddenly shortly after fistula construction prior to usage and attempts to create a functioning fistula in patients 18 and 20 were unsuccessful The fistula in patient 9 clotted after functioning for 7 1/ months

Initial usage

Initial use of the fistula in 16 patients (nos 1–3 5–13 15–17 21) varied from 5–111 days after construction In 2 patients (nos 4 and 19) the fistula has been functioning for 4 1/ to 15 1/ months respectively without being utilized Early utilization (<5 days) was frequently difficult because of edema over the autograft and poor initial arterialization with resultant low pressure and flow making venapuncture difficult

Function

Clinical and biochemical response to intermittent hemodialysis with the saphenous vein autograft A V fistula was similar to that observed with the Quinton–Scribner Teflon Silastic and Thomas® external A V cannula (2 4)

Emotional response

In general all patients who had previous external shunts accepted repeated venipunctures after a period of anxiety and recognized the advantages of the fistula (freedom of activity no clotting or infection) However in the younger children each venipuncture produced anxiety despite local anesthesia

The nursing staff preferred the external cannula to the fistula in the younger children because of venipuncture difficulty hematoma formation which occasionally delayed dialysis and anxiety in the younger children

Patient 7 who is on home dialysis performs venipunctures in the thigh A V fistula without assistance Similarly a number of adolescent patients have been taught to per

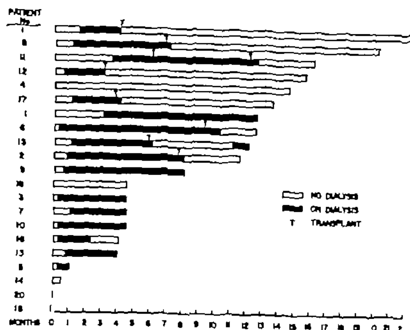


Fig 1 Fistula survival

form venipunctures in the dialysis unit without complications

Complications

The fistulas failed in 4 patients (nos 3 9 18 and 20) In patients 3 and 18 fistula flow ceased within 24 hours of construction because of the small size of the autograft A successful fistula was subsequently created in the opposite arm in patient 3 and insertion of an external shunt permitted dialysis in patient 18 The primary disease in patient 20 was lupus erythematosus and renal failure occurred secondary to renal vein and inferior vena cava thrombosis The fistula clotted shortly after construction and despite thrombectomy and the use of anticoagulants the fistula never functioned Inadequate function occurred in patient 9 because of scarring over the limbs of the fistula which militated against adequate venipuncture Clotting occurred after 7 1/ months presumably because of contracting scar tissue and the patient is at present being dialyzed with the use of a Thomas® shunt in the groin

Fistula flow ceased within 12 hours of surgery in patient 12 Re exploration revealed obstruction secondary to kinking of the autograft and revision was successful

No significant local or systemic infections occurred in any patient Infrequent hematoma formation resulted from maladroit venipuncture but in no instance did it delay dialysis for longer than 24 hours No evidence of ischemia to the distal extremity resulted from the fistula creation and no overgrowth of the involved extremity was evident clinically or radiologically Routine roentgenographs of the chest and electrocardiogram were obtained serially and no evidence of cardiac overload was observed

COMMENT

Our results utilizing the saphenous vein autograft A V fistula for extended hemodialysis in children has been satisfactory in contrast to the recommendation that subcutaneous fistulae should not be used in children (5) The advantages of the internal fistula for pediatric patients are 1) the absence of infection 2) rarity of clotting episodes and 3) freedom of arm movement We have preferentially used the saphenous vein autograft A V fistula although the side to side Brescia fistula (1) has been used successfully in children (7 12) A modification of the U shaped autograft fistula (10) was used successfully in 2 patients with crea

tion of a straight fistula anastomosing the autograft to the radial artery and cephalic vein This technique permitted utilization of more venipuncture sites

Fistula failure resulted from autograft clotting in 4 instances (17) which is higher than that reported in adult patients Kuruvila & Beven (5) reported a 9% incidence of thrombosis in a large series of patients with various fistula The small vessel size of children may account for the high incidence in our series Poor blood flow resulted in fistula failure from clotting in 2 patients (nos 3 and 18) In patient 20 thrombosis of the fistula occurred in a patient with a generalized occlusive vascular disease Similar phenomenon have been reported in adults (5 13) Early thrombosis requiring surgical intervention which occurred in patient 12 has also been reported frequently in adult patients (5 13) Clotting of the fistula after successful transplantation did not occur (8 15) in contrast to our experience with external cannula

No significant infectious complications occurred in our patients Kuruvila & Beven (5) reported the occurrence of infection with the development of a false aneurysm in 2 patients and Levi *et al* (6) reported 7 instances of septicemia in 6 patients with internal fistulas Pulmonary infarction resulted in 2 of the latter patients

Several reports have mentioned the hemodynamic effects of A V fistulas (1 3 5 7) and observed no significant abnormalities Similarly no evidence of cardiac decompensation occurred in our patients

Recently Matolo et al (8) reported ischemia neural damage affecting predominently the median nerve resulting from shunting of arterial blood in 2 patients with internal fistulas The paresthesia was relieved by surgical closure of the fistula No clinical evidence of neural ischemia was evident in any of our patients

Despite the paucity of complications and the beneficial effects of the saphenous vein autograft A V fistula in comparison with the external cannula we hesitate to enthusiastically recommend this type of shunt for younger children because of the continued anxiety raised by the requirement of repeated vena punctures The patency of the fistula after transplantation obviated the need for additional surgery in the event of allograft failure

We currently attempt to create an A V fistula in any patient with chronic renal failure who is potential candidate for cadaveric transplantation when the creatinine clearance approaches 5 ml/min/1 73 m

SUMMARY

Twenty two saphenous vein autograft arteriovenous (A V) fistulas were constructed in 21 patients aged $6^{a}/_{1}$ to $20^{a}/_{1}$ years Clotting resulted in fistula failure in 4 instances 16 fistulas have been utilized for repetitive hemodialysis for 1 to 11 months 2 fistulas have functioned for 4 ¹/ to 15 ¹/ months but have not as yet been utilized for dialysis and 1 patient died suddenly shortly after the fistula was created The advantages of the internal fistula for pediatric patients was the absence of infection rarity of clotting episodes and freedom of arm movement Despite the lack of complications and the advantages of the subcutaneous A V fistula in comparison with the external cannula general acceptance was not uniform in the younger children because of the requirement for repeated venipunctures

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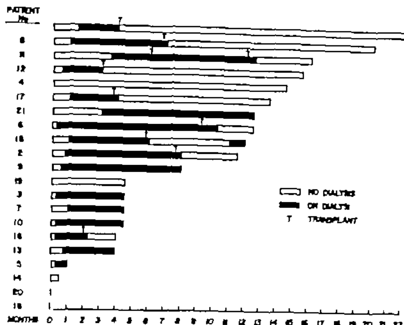


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Twenty two saphenous vein autograft arterio venous (A V) fistulas were constructed in 21 patients aged 6 $^{9}/_{12}$ to 20 $^{8}/_{12}$ years Clotting resulted in fistula failure in 4 instances 16 fistulas have been utilized for repetitive hemodialysis for 1 to 11 months 2 fistulas have functioned for 4 $^{1}/_{2}$ to 15 $^{1}/_{2}$ months but have not as yet been utilized for dialysis and 1 patient died suddenly shortly after the fistula was created The advantages of the internal fistula for pediatric patients was the absence of infection rarity of clotting episodes and freedom of arm movement Despite the lack of complications and the advantages of the subcutaneous A V fistula in comparison with the external cannula general acceptance was not uniform in the younger children because of the requirement for repeated venipunctures

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REQUIREMENTS FOR PROTEIN AND ESSENTIAL AMINO ACIDS IN EARLY INFANCY[1]

Studies with a Soy Isolate Formula

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Requirements for essential amino acids in early infancy are not yet firmly established Snyderman and co workers in a series of reports published between 1955 and 1964 provided estimates of requirements for individual essential amino acids based on studies in which mixtures of amino acids were fed to human infants These studies have been summarized by Holt & Snyderman (14 15) and by Fomon & Filer (6) The extent of relevance of such studies to requirements of infants fed whole proteins remains in serious question In addition we have pointed out (6) that in the studies of Snyderman and co workers nitrogen intakes were rather high (equivalent to protein intakes of approximately 3 g/kg/day) attempts to estimate the requirements for a particular amino acid were carried out with relatively few infants observations at a specified amino acid intake were of short duration and conclusions about adequacy of amino acid intake were based on short term rates of gain in weight and on nitrogen balance which may not be the most sensitive criteria

An alternate and we believe preferable approach to estimating requirements for essential amino acids consists of feeding whole proteins of known amino acid composition to normal infants over a period of several months Under these circumstances of study gain in length as well as gain in weight may be employed for evaluating growth rate and serum concentrations of albumin can be determined at intervals as an index of protein nutritional status (see Discussion) Such an approach was utilized in a study with a formula providing 1 65 g of cow milk protein per 100 kcal (6 12) The present study with a soy isolate formula was undertaken because it was evident that the formula chosen would supply lesser intakes of certain essential amino acids than those provided by the milk based formula employed in the previous study

SUBJECTS AND FEEDINGS

Normal fullterm female Caucasian infants with birth weights greater than 2 500 g were enrolled in the study during the first 9 days after birth The subjects included in the study represented all such female infants available for enrollment between October 1970 and January 1971 Nearly all were daughters of students or younger staff members of the University of Iowa Birth weight of each infant is indicated in Appendix I

During the first few days after birth nearly all of the infants were fed Similac[2] or Enfamil[3] and several of the infants received one of these formulas until 6 to 9 days of age Thereafter each infant was fed ad libitum experimental Formula 5224A prepared from soy isolate protein (Edi Pro A[5]) fortified with

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Table 1 *Composition of experimental soy isolate formula 5224A*

Major constituents (g/100 ml)			
Protein	1 10		
Fat	3 6		
Carbohydrate	7 5		
Ash	0 41		
L methionine (added)	0 005		
Content of minerals per liter			
Calcium	745 mg		
Phosphorus	489 mg		
Sodium	13 meq		
Potassium	18 meq		
Chloride	15 meq		
Magnesium	50 mg		
Iron	12 mg		
Zinc	2 59 mg		
Copper	0 69 mg		
Iodine	0 15 mg		
Content of vitamins per liter			
Vitamin A	1 638 I U		
Vitamin D	400 I U		
Vitamin E	5 I U		
Thiamin	967 μg		
Riboflavin	626 μg		
Niacin	6 500 μg		
Pyridoxine	538 μg		
Pantothenic acid	5 000 μg		
Folic acid	150 μg		
Vitamin B 12	3 μg		
Biotin	150 μg		
Ascorbic acid	82 mg		
Content of amino acids (mg)			
	per gram of protein[a]		*per 100 kcal of formula*
	mean	range	mg/100 kcal
Lysine	61	60–63	101
Histidine	24	23–27	40
Arginine	79	76–82	130
Aspartic Acid	125	123–128	206
Threonine	36	36–36	49
Serine	52	52–53	86
Glutamic Acid	209	203–213	345
Proline	55	52–60	91
Glycine	41	40–42	68
Alanine	40	38–42	66
Valine	50	49–51	83
Methionine	10	9–11	24[b]
Isoleucine	48	45–51	79
Leucine	80	77–83	132
Tyrosine	39	36–42	64
Phenylalanine	51	49–51	84
Cystine	15	15–15	25
Tryptophan	11		18

[a] Data from analyses of five lots of Edi Pro A
[b] Including 4 5 mg/g protein (5 mg/100 ml) added in manufacturing
Value provided by Ralston–Purina Company St Louis Missouri

L methionine[1] (5 mg/100 ml) a mixture of 58 corn and 42 coconut oils a mixture of equal parts by weight of sucrose and corn syrup solids (D E 18) and vitamins and minerals The chemical composition of this formula is presented in Table 1

Data concerning amino acid concentrations (Table 1) were supplied by Ross Laboratories from analyses of five lots of Edi Pro A Data on Edi Pro A provided by the supplier are in general agreement expressed in milligrams of amino acids per gram of protein these are lysine 57 histidine 23 threonine 35 valine 48 methionine 12 isoleucine 47 leucine 78 phenylalanine 54 tryptophan 11

At the time an infant was enrolled in the study her mother was interviewed by one of us (L N T) details of the program were outlined and written instructions were provided

The experimental formula was provided in ready to feed disposable glass bottles containing 120 or 240 ml The entire supply (approximately 13 000 bottles) was prepared by the manufacturer as a single batch A supply of formula sufficient for 72 or 96 hours was weighed and delivered to the family When a new supply was delivered 3 or 4 days later the bottles from the previous supply including any unconsumed amounts of formula were collected and again weighed Bottles for each 24 hour period were weighed separately

During the first 28 days of life the formula served as the sole source of nutrients Thereafter the infants were permitted to receive commercially prepared strained foods from one manufacturer[a] according to the following schedule at age 28 days oatmeal with applesauce and bananas at 56 days pears and at 84 days (two foods) applesauce and bananas with tapioca The proximate composition and density of each strained food has been presented previously (13) Caloric densities of the four foods are 77 68 87 and 85 kcal per 100 g respectively Corresponding protein concentrations are 1 5 0 3 0 2 and 0 5 g/100 g Parents were advised that addition of such foods to the diet was optional and that the formula was a complete food Empty (or partially empty) jars were collected at 3 or 4 day intervals and weighed From the weight of strained food consumed and its density volume of intake of each of these strained foods was calculated

PROCEDURES AND METHODS

We have described previously (13) the manner in which infants in our studies are weighed and measured

Blood for serum chemical determinations was obtained by venipuncture of the external or internal jugular vein With few exceptions blood was obtained between 1 00 and 1 30 pm with no restriction relating to the time of feeding The quantity of blood

Nutritional Biochemicals Corp Cleveland Ohio
Ralston Purina Company Data sheet 10-4A
Gerber Products Company Fremont Michigan

obtained was approximately 6 ml Only those serum chemical determinations reflecting protein intake (concentration of urea nitrogen) or protein nutritional status (total protein and albumin) are reported here Concentration of urea nitrogen was determined by the carbamido-diacetyl reaction modified for autoanalysis (18 2° a) Concentration of total protein was determined by a minor modification (22 b) of the automated biuret reaction as described by Failing et al (3) Concentration of albumin was calculated from the percentage of total protein accounted for by albumin as determined by electrophoresis on cellulose acetate as described previously (11)

Formula urine and feces were digested by the micro-Kjeldahl method (16 a) using selenium rather than mercuric oxide as a catalyst Nitrogen concentration of the digested mixture was determined by microdiffusion (2) Nitrogen concentrations of strained foods were determined by the Dumas procedure (16 b) with the use of a nitrogen analyzer With the exception of cystine which was determined by the method of Schram et al (21) amino acid concentrations of Edi Pro A were determined by the method of Moore et al (19)

The manufacturer's stated analyses was utilized for concentration of fat in formula and strained foods

At the completion of the growth study 6 of the infants served for metabolic balance studies one balance study being performed with each infant in the manner previously described (8 a) Concentration of fat in feces was estimated by the method of Van de Kamer et al (17) which we have found to yield results similar to those obtained with a gravimetric method employing fat extraction (9)

INTERVALS OF STUDY

In describing size of the infants (e g Appendix I) recorded measurements were "corrected" by parabolic interpolation or extrapolation utilizing three adjacent values to reflect values applicable to ages 8 14 28 42 56 84 and 112 days For convenience these age designations have been employed throughout

The day on which measurements of length and weight were made was employed as the first day of the interval for recording food intake Thus volume of intake and calorie intake are presented (Table 2) for ages 8 through 41 (8–41) 42 through 111 (42 111) or 8 through 111 (8–111) days Gains in length and weight (Table 3) on the other hand are expressed as 8 to 42 (8–42) 42 to 112 (42 112) or 8 to 112 (8–112) days In the case of discussions involving both food intake data and gains in weight the designations 8–41 42 111 or 8–111 days are used

Nitrogen Analyzer Model 29B Coleman Instruments, Inc Maywood Illinois

The amino acid analyses were performed by Mrs Helen Chrtella Ross Laboratories Columbus, Ohio using a Beckman Amino Acid Analyzer Model 120 (Beckman Instrument Company Fullerton, Cali

Table 2 *Mean calorie and protein intakes from 8 through 111 days of age by normal female infants fed a soy isolate formula (5224A) or a milk based formula (29B)[a] supplying similar quantities of protein*

	Formula 5224A (13 subjects)		Formula 29B (8 subjects)	
	Mean	S D[b]	Mean	S D
Calorie intake				
8–41 days				
kcal/day	432	81	417	41
kcal/kg/day	113	10	102	13
42–111 days				
kcal/day	542	68	517	44
kcal/kg/day	105	9	95	5
8–111 days				
kcal/day	506	61	484	35
kcal/kg/day	107	10	98	6
Protein intake				
8–41 days				
g/day	7 1[illegible]	1 35	7 48	0 73
g/kg/day	1 86	0 33	1 84	0 22
g/100 kcal	1 65	0 01	1 80	0 01
42–111 days				
g/day	8 68	1 04	9 1	0 82
g/kg/day	1 68	0 15	1 69	0 10
g/100 kcal	1 61	0 02	1 76	0 03
8–111 days				
g/day	8 17	0 96	8 58	0 70
g/kg/day	1 74	0 16	1 74	0 11
g/100 kcal	1 62	0 02	1 77	0 02

[a] Data of Fomon et al (6 13)
[b] Standard deviation

Mean values for body weight during the intervals 8–42 and 42 112 days are employed in certain of the calculations presented in the Discussion Mean body weight for the 34-day interval from 8 to 42 days of age was estimated as follows mean weight for the interval 8–14 days was assumed to be the sum of the weight at 8 days and the weight at 14 days divided by 2 similarly the mean weight for the interval 14–28 days and for the interval 28–42 days was calculated The mean weight for the entire interval 8–42 days was then computed by weighting the values for the shorter intervals according to the relative durations of the intervals (i e 6 14 and 14 days respectively) Mean body weights during the intervals 42 112 days and 8–112 days were estimated in a similar manner

RESULTS

Fifteen infants were enrolled in the study and 13 completed the planned interval of observation from 8 to 112 days of age One infant (Subject 1527) developed diarrhea and was

Table 3 *Mean gains in weight and length between 8 and 112 days of age by normal female infants receiving various feedings*

		Age 8–42 days			Age 42–112 days			Age 8–112 days		
		Gain in weight		Gain in length	Gain in weight		Gain in length	Gain in weight		Gain in length
	No of subjects	(g/day)	(g/100 kcal)	(mm/day)	(g/day)	(g/100 kcal)	(mm/day)	(g/day)	(g/100 kcal)	(mm/day)
Formula 5224A	13	30 4 (6 0)[a]	7 12 (1 25)	1 26 (0 22)	23 2 (4 0)	4 26 (0 36)	0 92 (0 14)	25 5 (3 8)	5 05 (0 50)	1 03 (0 09)
Formula 29B[b]	8	29 8 (6 3)	7 09 (1 05)	1 18 (0 20)	22 5 (4 9)	4 34 (0 79)	0 92 (0 16)	24 9 (4 9)	5 12 (0 78)	1 00 (0 13)
Breastfed										
First series	46	33 1 (7 7)		1 27 (0 17)	22 5 (5 5)		0 91 (0 14)	26 0 (4 7)		1 03 (0 12)
Second series[d]	44	35 2 (9 2)		1 24 (0 18)	22 4 (5 8)		0 90 (0 12)	26 6 (5 4)		1 02 (0 09)

[a] Data in parentheses indicate standard deviations
[b] Data of Fomon et al (3 5)
[c] Data of Fomon et al (6)
[d] Unpublished—see text

withdrawn from study on the advice of her pediatrician Subject 1535 was hospitalized because of ventricular septal defect These infants were studied until 56 days of age Data on weight and length of each infant at various ages and on food intake of each infant in various age intervals are presented in Appendices II and III From these data performance of Subjects 1527 and 1535 may be compared with that of the other infants It will be found that food intake and gains in weight and length through 56 days of age were similar for the 13 infants who remained in the study until 112 days of age and for the 2 infants who failed to complete the study Biochemical values in serum were also similar Data presented in Tables 2–4 of this report are limited to observations of the 13 infants who completed the planned period of observation from 8 to 112 days of age

For purposes of evaluating results of the present study we have utilized our published data concerning female breastfed infants (11) In addition to these published data (first breastfed series in Table 3) we have included more recent unpublished data (second breastfed series in Tables 3 and 4) concerning female breastfed infants managed in a similar fashion For reasons to be presented (see *Serum Concentrations of Urea Nitrogen and Proteins*) we consider the second breastfed series to be more satisfactory than the first as a reference for evaluation of serum concentration of albumin

Intake of calories protein and amino acids

During the age intervals 8–41 42–111 and 8–111 days intakes of calories whether expressed as kcal/day or as kcal/kg/day were slightly greater by infants fed the soy isolate Formula 5224A than by infants fed milk based Formula 29B in our previous study (Table 2) Mean protein intakes when expressed as g/day were less by infants fed Formula 5224A than by those fed Formula 29B but when expressed as g/kg/day were nearly identical In each age interval the ratio of protein intake to calorie intake was slightly less by infants fed Formula 5224A than by those fed Formula 29B With each feeding the slightly lower ratio of protein intake to calorie intake during the age interval 42–111 days than during the age interval 8–41 days reflects the greater consumption of low protein strained foods by older infants

In the age interval 8–41 days, consumption

Table 4 *Serum urea nitrogen total protein and albumin of normal female infants fed soy isolate formula 5224A or breastfed*[a]

	28 days		56 days		84 days		112 days	
	Formula 5224A	Breast fed	Formula 5224A	Breast fed	Formula 5224A	Breast fed	Formula 5224A	Breast fed
Urea nitrogen (mg/100 ml)								
Mean	6.3	7.6	6.4	6.6	6.3	6.4	6.8	6.2
S D	1.3	2.1	1.1	2.0	1.4	1.9	2.0	3.0
No of subjects	7	31	12	31	11	34	12	32
Total protein (g/100 ml)								
Mean	5.2	5.3	5.4	5.5	5.4	5.5	5.8	5.8
S D	0.3	0.4	0.7	0.5	0.4	0.4	0.4	0.5
No of subjects	8	26	12	28	11	30	12	27
Albumin (g/100 ml)								
Mean	3.4	3.4	3.7	3.6	3.8	3.7	4.1	3.9
S D	0.2	0.2	0.4	0.4	0.4	0.4	0.4	0.3
No of subjects	8	26	12	28	11	30	12	27

[a] Second series Unpublished—see text

of strained food (oatmeal with applesauce and bananas) accounted for an average of 2.7 of total intake of calories and for an average of 3.1 of total intake of protein In no instance did the strained food account for more than 6.7% of total intake of protein In the age interval 42–111 days consumption of the four strained foods permitted during some or all of the interval averaged 9.6 and 7.3 respectively of total intakes of calories and protein Protein obtained *from* strained foods during this interval accounted for 13.7% 11.8 and 10.5% respectively of total protein intakes of Subjects 1530 1523 and 1525 and for less than 10 of total protein intake of each other subject

During the entire interval 8–111 days calorie and protein intakes from strained foods averaged 7.7% and 6.1% respectively *of total* intakes of calories and protein In only one instance did intake of protein from strained foods exceed 10% of total protein intake (Subject 1530 11.7% of protein intake from strained foods)

Because intake of protein from strained foods accounted for a relatively small percentage of total protein intake and because the amino acid composition of oatmeal protein (the major protein contributed by the strained foods) is rather similar to that of the soy isolate protein Edi Pro A we have assumed that the ratio of amino acid to protein in the total diet was the same as that of the soy isolate protein (Table 1) That this assumption does not introduce important errors is apparent from the following consideration of intakes of individual amino acids

As indicated in Table 1 the soy isolate formula provides 50 mg of valine per gram of protein Oatmeal provides 59 mg of valine per gram of protein (20) If 10% of protein intake were obtained from oatmeal and the remainder from the soy isolate formula the intake of valine per gram of protein of the total diet would be 51 mg—a value only 2% different from the value that would have been obtained had all the protein been derived from soy isolate In the case of lysine if 10% of protein intake were supplied by oatmeal protein (33 mg of lysine per gram of *protein (20)*) and the remainder from the soy isolate protein the resultant intake of lysine would be 58 mg of protein compared with the value of 61 mg per gram of protein in the soy isolate

Table 5 *Three day metabolic balance studies with normal female infants fed soy isolate formula 5224A*

Subject	Age (days)	Weight (g)	Length (cm)	Nitrogen (mg/kg/day) Intake	Excretion Urine	Feces	Total	Retention	Fat (g/kg/day) Intake	Excretion
1521	116	6 690	60 8	302	124	58	182	120	6 22	0 40
1524	113	5 465	60 9	298	122	61	183	115	6 13	0 54
1525	114	6 665	63 2	198	89	46	135	63	4 07	0 41
1526	118	5 430	58 9	271	112	42	154	117	5 57	0 54
1529	117	5 265	57 4	300	126	49	175	125	6 17	0 65
1533	114	5 120	61 1	208	84	52	137	71	4 27	0 44

protein With the exceptions of valine and lysine the difference in concentrations of any essential amino acid in oatmeal does not differ from that in the soy isolate protein by more than 6 mg per gram of protein

Gain in weight and length

As may be seen from Table 3 mean gains in weight and length by infants fed Formula 5224A were similar to those observed previously in studies of infants fed Formula 29B and in studies of breastfed infants Weight gain per 100 kcal consumed was similar for infants fed Formula 5224A and for those fed Formula 29B

Serum concentration of urea nitrogen and proteins

Concentrations of urea nitrogen in serum of normal infants receiving adequate calorie intakes reflect the intakes of nitrogen (8 b) Thus, the similar serum concentrations of urea nitrogen by breastfed infants (Table 4) and infants fed Formula 5224A suggest that intakes of protein were similar These values are also similar to those we have reported previously for breastfed infants (11)

Data concerning serum concentrations of total protein must be interpreted in relation to the method employed As we have discussed previously (12) the biuret method performed in the customary manner without a serum blank appears to be satisfactory for analysis of serum obtained from fasting individuals However in our studies of infants, blood is obtained without regard to time of feeding and lipemic serum may be responsible for falsely high biuret values We therefore consider that concentrations of total protein and concentrations of albumin which are dependent on concentrations of total protein were falsely high (perhaps 10% high) in our published reports concerning breastfed infants (11) and infants fed Formula 29B (6 12) The data presented in Table 4 concern total protein concentrations determined by an automated method employing a serum blank It may be seen that concentrations of total protein and albumin were similar for infants fed Formula 5224A and for breastfed infants

Metabolic balance studies

Results of three day metabolic balance studies with six infants between *113 and 118 days* of age are presented in Table 5 and Fig 1 As may be seen from Fig 1 the relation between intake and excretion of nitrogen in studies with infants fed Formula 5224A was similar to that reported in our previous studies of 90- to 120-day old infants fed human milk or Formula 29B

Included in Table 5 are data concerning intakes and excretions of fat by infants fed Formula 5224A Excretions of fat ranged from 6 4 to 10 5 of intake values similar to those we have found for excretion of fat when mixtures of corn and coconut oils are fed in milk based formulas (10)

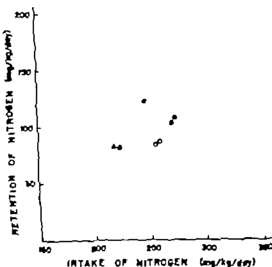


Fig 1 Retention of nitrogen versus intake of nitrogen by normal infants between 90 and 120 days of age Each point refers to results of one 3 day metabolic balance study Each refers to a study of an infant fed the soy isolate Formula 5224A described in this report The other symbols refer to our earlier observations ▲ infants fed milk based Formula 278 (6 12) ○ infants fed fresh human milk (4) ● infants fed processed human milk (5)

DISCUSSION

In the present study 13 female infants were fed ad libitum an L methionine fortified soy isolate formula providing 1 1 g of protein and 67 kcal per 100 ml In addition they were permitted to receive certain low protein strained foods with the result that the total diet provided approximately 1 62 g of protein per 100 kcal The infants appeared in good health during the period of study from 8 to 112 days of age gains in length and weight and serum concentrations of albumin were similar to those of normal breastfed infants Nitrogen balance data concerning 6 of the infants were similar to corresponding data concerning infants fed fresh or processed human milk

On the basis of the satisfactory growth and maintenance of normal serum concentrations of albumin we conclude that under the conditions of these studies the requirement for protein of normal female infants between 8 and 112 days of age is no greater than 1 62 g of protein per 100 kcal We do not consider that nitrogen balance studies constitute a useful index of protein nutritional status under conditions similar to those of the present study The nitrogen balance data are included for the scrutiny of those who do not share our view on this point Whether serum concentration of albumin is a sensitive or insensitive index of protein nutritional status remains a matter of controversy (1 23) Most of the data on serum concentrations of albumin published before 1960 were obtained by salting out methods of low reliability Differences in albumin concentrations between groups might well have been obscured by the wide variability within groups With current widespread use of electrophoretic methods for protein separation serum concentrations of albumin may prove to be a useful index of protein nutritional status In the present study we can only state that during the 3½ months of observation it was impossible to detect any difference in serum concentrations of albumin between healthy breastfed infants and those fed the experimental diet

On the basis of data included in the present report together with our previous observations concerning female infants fed a diet providing approximately 1 77 g of protein per 100 kcal primarily from cow milk we conclude that under conditions of these studies the requirements for amino acids per 100 kcal are no greater than the values indicated in Table 6

It seems desirable to comment further on the proviso under the conditions of this study and to present our reasons for preferring to express requirements for protein and amino acids in terms of quantity per unit of calorie intake rather than in the more commonly employed terms of quantity per kilogram of body weight

Any estimate of requirement applies directly only to those circumstances under which the estimate was made Extension to other circumstances must be made with caution The present study suggests that when high quality protein is fed to normal female infants who

Table 6 *Estimated requirements for amino acids by infants*

Amino acid	Observed intake (mg/100 kcal)		Estimate of requirement[a] (mg/100 kcal)	Estimate of requirement (mg/kg/day)	Ranges of observed intake (mg/kg/day)	
	Fomon & Filer (3) (milk based formula 29B)	Present study (soy isolate formula 5224A)	Fomon & Filer (3) and present study	Holt & Snyderman (1 2)	Fomon & Filer (3)	Present study
Histidine	26	40	26	16–34	21–32	39 52
Isoleucine	66	79	66	102–119	54–81	78–102
Leucine	152	132	132	76–229	125–185	130–171
Lysine	152	101	101	88–102	125–185	99 131
Phenylalanine	57	84	57	47–90	47–70	82 109
Methionine	28	24	24	33–45	23–24	24–31
Cystine	23	25	23		19–28	25–32
Threonine	110	59	59	45–87	90–134	58–77
Tryptophan	16	18	16	15–22	13–20	18–23
Valine	88	83	83	85–105	72–107	82 108

[a] Although the actual requirements under these conditions of study are unlikely to be greater than the values listed they may in some instances be substantially less than the indicated values

receive adequate intakes of calories and of non nitrogenous essential nutrients the requirement for protein is no greater than 1 62 g per 100 kcal Although our study with the cow milk protein Formula 29B was performed at a slightly higher ratio of protein to calories (1 77 g protein per 100 kcal) there is little reason to suspect that the protein requirement would be greater with cow milk protein than with L methionine fortified soy isolate protein We speculate that 1 62 g of high quality protein per 100 kcal in an otherwise satisfactory diet will be found adequate whether the protein source is L methionine fortified soy isolate human milk cow milk egg meat or fish We suspect that the requirement for protein per 100 kcal is not remarkably different for males and for females and, in fact, in our previous study with Formula 29B performance by both males and females was satisfactory with an intake of protein of 1 77 g/100 kcal Finally we speculate that when a whole protein (rather than a mixture of amino acids) is fed in amounts not greatly exceeding the requirement and in the presence of adequate calories and of non nitrogenous essential nutrients requirements for essential amino acids per 100 kcal will be found to be no greater than those indicated in Table 6

Our preference for expressing requirements for protein (and amino acids) per unit of calorie intake rather than per unit of body weight is restricted to infants In the case of older children and adults most of the requirement for protein is utilized for repair or replacement (maintenance) only a relatively small fraction being utilized for synthesis of new tissue (growth) Because maintenance requirements are related to body size it is reasonable to express requirement for protein in relation to body weight whenever maintenance accounts for a high proportion of total requirement However when requirement for growth accounts for a large percentage of total requirement as is the case during infancy expression of requirement per unit of body weight is less meaningful We believe that calorie intake which reflects both size and rate of growth (13) is a more satisfactory unit of reference than is body weight

An example of the advantage of expressing requirement per unit of body weight is provided by comparison of the performance of the most rapidly growing infants fed Formula 5224A with that of the least rapidly growing infants fed this formula During the period 8 through 41 days, 3 infants (Subjects 1524 1526 and 1531) gained less than 27 0 g/day

their mean gain in weight was 23 8 g/day[1] mean calorie intake 338 kcal/day and mean protein intake 5 52 g/day Three other infants (Subjects 1522 1523 and 1530) gained more than 38 0 g/day during the same interval with mean gain of 39 0 g/day mean calorie intake of 502 kcal/day and mean protein intake of 8 37 g/day In relation to body weight mean intake of protein of the three most slowly growing infants was 1 57 g/kg/day whereas that of the three most rapidly growing infants was 2 01 g/kg/day Yet in each instance 1 65 g of protein per 100 kcal (Table 2) was judged adequate Similar considerations apply when expressing requirements for essential amino acids Thus by expressing requirements for protein or for essential amino acids in terms of quantity per 100 kcal we believe that individual differences between infants of a specified age and weight and between male and female infants will to some extent be minimized

For several reasons one might anticipate that among normal infants protein requirements per 100 kcal would be greater for younger than for older subjects Protein requirement per unit of calorie intake is almost certainly greater for synthesis of new tissue (growth) than for maintenance (repair or replacement) younger infants grow more rapidly than do older infants and older infants with their larger body size utilize a greater percentage of calories for maintenance If we consider the period from 8 to 42 days of age when growth is most rapid gain in weight by infants fed Formula 5224A averaged 30 4 g/day (Table 3) mean body weight was 3 828 g and protein intake averaged 7 12 g/day (Table 2) Assuming that the increment in body weight contained 11 4 protein (7) 3 47 g of protein daily were needed for growth Because the total requirement for protein to accomplish this mean gain in weight of 30 4 g/day during the interval 8 through 41 days was presumably no greater than the 7 12 g/day consumed the requirement for maintenance appeared to be no greater than 3 65 (i e 7 12 minus 3 47) g/day or 0 95 g/kg/day

Assuming that the requirement for protein for maintenance is the same per unit of body weight (i e 0 95 g/kg/day) during the age interval 42 through 111 days as during the age interval 8 through 41 days and that protein comprises 11 4% of the increment in body weight (7) one may estimate the total requirement for protein for the interval 42 through 111 days of age The average weight gain during this interval was 23 2 g/day (Table 3) average body weight 5 206 g and average calorie intake 542 kcal/day (Table 2) The calculated protein requirement for growth was 2 65 g/day (23 2 g/day × 11 4) and that for maintenance was 4 77 g/day (5 2 kg × 0 95 g protein/kg/day) for a total requirement of 7 42 g/day This amounts to 1 37 g protein per 100 kcal Thus if as we suppose the requirement for protein is approximately 1 6 g/100 kcal for the interval 8 through 41 days of age it may be 1 4 g/100 kcal for the interval 42 through 111 days of age

It must be emphasized that our preliminary estimates of requirements for individual essential amino acids (Table 6) have been obtained by an approach that is likely to lead to over estimation of requirements Satisfactory performance of infants under the particular conditions of study suggests that requirements for protein and essential amino acids were *no greater than* the amounts consumed It is of course likely that requirements for some amino acids will be substantially *less* than the amounts consumed

In the present study concentrations of leucine lysine methionine and tryptophan were less per 100 kcal and concentrations of histidine isoleucine and phenylalanine were

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[1] This value is only slightly more than one standard deviation below the mean for female breastfed infants (Table 3)

[2] The method of computing mean body weight has been described (see Procedures and Methods) However it will be apparent that the reader can divide the value in Table 2 for calorie intake expressed as kcal/day by the corresponding value expressed as kcal/kg/day to obtain mean body weight.

greater per 100 kcal than in our previous study (6) with the milk based Formula 29B (Table 6) Concentrations of methionine, cystine tryptophan and valine per 100 kcal were not remarkably different in the two studies Because of the satisfactory performance of the infants we presume that intakes of essential amino acids were adequate in both studies and have selected as our preliminary estimate of requirement for each amino acid the lower concentration per 100 kcal

Because the estimates of requirement for essential amino acids summarized by Holt & Snyderman (14 15) are expressed per unit of body weight we have included in Table 6 the observed intakes of amino acids expressed as mg/kg/day In presenting the range of intakes of each amino acid observed in our study with milk based Formula 29B (6) we have utilized the data on calorie intakes per kilogram pertaining to all 18 infants (males and females) fed this formula from 8 through 111 days of age The calorie intake per kilogram of the infant with the least calorie intake per kilogram has been multiplied by the amino acid intake per 100 kcal (Table 6) to give the value presented as the low point of the range of amino acid intake expressed as mg/kg/day In similar manner the upper value of the range has been calculated for infants fed milk based Formula 29B and the lower and upper values of the range for infants fed soy isolate based Formula 5224A

The estimates of requirements by Holt & Snyderman (14 15) represent the least intakes at which the infants were judged to be performing satisfactorily with respect to shortterm weight gain and nitrogen balance Thus, the isoleucine requirement of every infant was considered to be 102 mg/kg/day or more (Table 6) In our studies on the other hand intakes of isoleucine were 81 mg/kg/day or less in all 18 infants fed milk based Formula 29B and were less than 102 mg/kg/day in all but 1 of the 13 infants fed soy isolate Formula 5224A The least intake of methionine (33 mg/kg/day) considered satisfactory by Holt & Snyderman was greater than that of any infant in the present study with Formula 5224A and greater than that of 16 of 18 infants in our previous study with Formula 29B

Our findings with respect to isoleucine and methionine raise considerable doubt about the relevance of the estimates of Holt & Snyderman with respect to situations in which whole proteins are fed in amounts not greatly exceeding the protein requirement

SUMMARY

Thirteen normal female infants were observed from 8 through 111 days of age while receiving a diet providing 1 62 g of protein per 100 kcal almost entirely from soy isolate Clinical observations growth rates and serum concentrations of albumin were similar to those of female infants fed milk based formulas providing greater intakes of protein On the basis of these findings it is assumed that the requirements for protein and essential amino acids of these infants were no greater than the amounts consumed Reasons for preferring to express requirements for proteins and amino acids per unit of calorie intake rather than per unit of body weight are presented

The preliminary estimates of requirements presented here are believed applicable when the diet is adequate in total calories and non nitrogenous essential nutrients nitrogen is provided primarily in the form of whole proteins and protein intakes do not greatly exceed the requirement For reasons discussed the approach is likely to yield estimates of requirements for some amino acids that are substantially greater than the true requirements Nevertheless our estimates of requirements for isoleucine and methionine are distinctly less than those reported by Holt & Snyderman We conclude that the estimates of Holt & Snyderman from studies of infants fed mixtures of amino acids are less relevant than our estimates to circumstances in which whole proteins are fed in amounts that do not greatly exceed the requirement for protein

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Appendix I Weights and lengths of individual female infants fed Formula 5224A at various ages

Subject number	Birth	8 days	14 days	28 days	42 days	56 days	84 days	112 days
Weight (g)								
1521	3 630	3 690	3 821	4 230	4 619	4 977	5 988	6 733
1522	4 280	4 369	4 645	5 252	5 687	6 002	6 516	7 253
1523	2 950	2 975	3 049	3 580	4 285	4 851	5 919	6 498
1524	3 100	3 273	3 312	3 708	3 919	4 377	4 747	5 406
1525	4 070	3 875	4 157	4 645	5 011	5 470	6 163	6 651
1526	2 960	2 980	3 079	3 487	3 855	4 068	4 848	5 438
1527	3 390	3 186	3 364	3 720	4 154	4 235	—	—
1528	3 230	3 126	3 275	3 755	4 150	4 360	5 093	5 553
1529	2 930	2 881	3 025	3 480	3 890	4 300	4 698	5 322
1530	3 400	3 170	3 475	4 035	4 520	5 000	5 825	6 317
1531	3 250	3 168	3 202	3 610	4 075	4 330	5 056	5 575
1532	3 690	3 561	3 515	4 005	4 449	4 733	5 402	5 928
1533	2 850	2 798	3 090	3 573	3 877	4 169	4 591	5 063
1534	3 480	3 360	3 245	3 852	4 308	4 529	5 437	5 978
1535	3 280	3 380	3 479	3 594	3 735	3 870	—	—
Length (cm)								
1521		51 5	52 6	54 6	56 3	57 1	60 1	60 8
1522		52 1	53 4	56 5	57 3	59 1	61 7	64 2
1523		50 9	51 4	53 2	54 5	56 6	59 7	63 1
1524		50 8	51 7	52 4	54 7	56 4	58 7	60 8
1525		52 0	53 2	54 8	56 1	57 6	60 5	63 1
1526		49 0	49 6	51 2	53 1	54 4	55 9	58 5
1527		50 0	50 6	52 3	53 8	54 7	—	—
1528		49 3	50 0	51 2	54 0	55 2	58 0	60 8
1529		47 2	47 9	49 5	50 8	52 8	55 4	57 3
1530		51 1	51 2	54 8	56 2	57 4	59 5	62 4
1531		50 9	51 4	53 5	54 3	56 1	58 5	61 3
1532		52 3	53 2	54 4	55 4	56 8	58 9	62 0
1533		49 6	51 9	53 1	54 7	56 3	58 2	60 9
1534		51 7	52 1	53 6	56 7	57 1	59 5	62 5
1535		50 8	51 4	53 4	54 4	55 5	—	—

Appendix II Average daily weight (g) of Formula 5224A consumed by individual female infants during successive age intervals

Subject number	Age interval (days)					
	8–13	14–27	28–41	42–55	56–83	84–111
1521	436	466	611	832	944	978
1522	896	867	750	755	755	819
1523	467	764	857	946	882	736
1524	330	446	640	684	735	794
1525	434	684	676	719	714	694
1526	330	417	512	602	707	680
1527	338	399	590	485	—	—
1528	457	669	693	720	672	642
1529	475	504	731	713	649	746
1530	385	592	676	630	667	690
1531	504	549	585	591	674	674
1532	618	690	736	732	710	771
1533	464	680	591	623	622	575
1534	649	815	804	784	909	675
1535	604	546	557	526	—	—

Appendix III Average daily weight (g) of strained foods consumed by individual infants

Age interval (days)	Oatmeal with applesauce and bananas				Pears		Applesauce	Bananas with tapioca
	28–41	42–55	56–83	84–111	56–83	84–111	84–111	84–111
Subject no								
1521	—	40	28	17	—	21	18	8
1522	34	58	30	47	21	36	24	41
1523	69	90	45	76	30	72	29	44
1524	12	10	13	16	13	22	11	22
1525	49	46	39	58	54	46	24	27
1526	—	—	—	57	—	17	5	5
1527	33	21	—	—	—	—	—	—
1528	82	21	13	56	—	24	—	23
1529	—	—	—	—	—	—	—	—
1530	75	146	45	50	48	40	43	20
1531	17	20	33	44	24	44	13	17
1532	74	53	27	19	6	6	6	9
1533	15	17	24	70	21	23	8	41
1534	43	22	30	40	31	4	13	36
1535	15	13	—	—	—	—	—	—

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HOMOVANILLIC ACID AND 5 HYDROXYINDOLEACETIC ACID IN CEREBROSPINAL FLUID OF A CHILD WITH FAMILIAL DYSAUTONOMIA

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For many years the concentrations of homo vanillic acid (HVA) and 5 hydroxyindoleacetic acid (5 HIAA) have been determined in cere brospinal fluid (CSF) of various neurological conditions in childhood A case of familial dysautonomia which we investigated showed CSF values of HVA and 5 HIAA worth while reporting

CASE HISTORY

The patient is a six year old girl observed since birth mainly because of hypotonia but also fills the following criteria of familial dysautonomia (7) (*a*) Feeding difficulties during infancy (*b*) No visible lacrimation (*c*) No corneal reflexes (*d*) Pronounced postural hypotension and absence of deep tendon reflexes (*e*) Pronounced emotional lability (*f*) In difference to pain and temperature (*g*) No fungiform papillae on the tongue (*h*) Absence of flare with intradermal histamine

She has been very susceptible to infections but has also shown periods of high temperature without any concomitant infection Her motor development is retarded but the mental capacity is normal for age Motor nerve conduction velocity was normal On repeated attempts to determine sensory nerve conduc tion velocity no responses at all could be elicited The parents are not of Jewish extraction and there are no other cases reported in the family

Lumbar puncture was first performed at the age of 3 / years Next lumbar puncture was made at the age of 4 / years A probenecid test was done *ad modum* Olsson & Roos (6) by giving 250 mg pro benecid orally twice daily for 2 days and the fifth dose on the third day 3 hours before lumbar punc ture Probenecid blocks the out transport of 5 HIAA and HVA from CNS and CSF to blood which is why it is used for studying the turnover of the cor responding amines in the brain The results of the 5 HIAA and HVA determinations are shown in Table 1

DISCUSSION

The 5 HIAA values in the present case are within the normal range for age (2) In our control material which comprises children with various neurological disorders, we do not have in this age group HVA values ex ceeding 100 ng per ml The mean value of HVA is 31 ± 12 (S D) ng per ml in young healthy volunteers (4)

It has been reported that patients with familial dysautonomia have a deficiency in the peripheral noradrenergic system mani fested in low outflow of vanillylmandelic acid (VMA) and relatively high HVA values in the urine (10) As a consequence these children react to adrenaline with a rapid rise in blood pressure apparently because of hypersensitivity of the noradrenergic receptors The finding in our case of high values of HVA in CSF could be due to an impairment of dopamine β hydroxylase (DBH) the enzyme transforming dopamine (DA) to noradrenaline (NA) Re cently Weinshilboum & Axelrod (11) has found a very low plasma concentration or no DBH at all in plasma from children wit

Table I *The values of 5 HIAA and HVA in CSF before and after probenecid test (expressed as ng/ml CSF)*

Age	5-HIAA	HVA
3 5 years	56	192
4.5 years before probenecid	n d	147
4 5 years after probenecid	86	166

familial dysautonomia A decrease in the enzyme activity of DBH will cause an increase in the intraneuronal DA concentration which results in an increase in the metabolites of DA, when the stores are filled and at the same time the NA concentration will decrease The rise in HVA after probenecid was in our patient 19 ng per ml or 13 of the basic value The increase in HVA after probenecid in normal adults is about 200 % (8) but considering the possible shifting in the quotient between DA and NA in our patient this way of calculating might be erroneous However a rise in DA due to the suggested shortage of DBH is likely to cause an impaired release and thus also synthesis of DA because of a feed back mechanism known from many other adrenergic processes of transmission (3) The slight increase of HVA after probenecid might in turn reflect this

A change in the elimination rate of HVA from CSF seems unlikely in view of the normal response of 5 HIAA after probenecid because it is known that these two acids use the same active transport mechanism (5) There is another factor which could contribute to a high HVA level in lumbar CSF Normally there is no DA and thus no HVA in the spinal cord (1) This might depend on the absence of storage sites for DA e g of granules not containing DBH If the transformation from "precursor DA to NA is disturbed also in the spinal cord this could cause accumulation of HVA in the organ and an inflow of HVA into the surrounding CSF

Nearly all symptoms in familial dysautonomia could be due to an imbalance between the adrenergic and the cholinergic systems caused by a defect in DBH activity both centrally and peripherally The findings in our case support that hypothesis

SUMMARY

A case of familial dysautonomia with a high level of HVA and a normal level of 5 HIAA in CSF is described After probenecid loading test the increase in HVA was only slight while the increase in 5 HIAA was normal These findings suggest an explanation for the pathogenesis of the disease

ACKNOWLEDGEMENTS

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PERIPHERAL CIRCULATORY RESPONSE TO PHOTOTHERAPY IN NEWBORN INFANTS

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In a previous report (6) we have documented changes in water balance in newborn infants who were treated by phototherapy because of jaundice These changes include significant in crease in stool water loss and insensible water loss during phototherapy The latter was as sociated with an increase in the respiratory rate It was postulated that the effects of radiant heat and/or conversion of photo-energy to heat energy produce changes in peripheral circulation initiated by some ther moregulatory mechanism This study was de signed to explore changes in peripheral cir culation and temperature to account for the increased insensible water loss

The use of phototherapy in the treatment of neonatal hyperbilirubinemia is currently in wide use (1 5) It seems pertinent that studies to explore significant clinical observations such as insensible water loss peripheral circulation and temperature changes should be performed

MATERIAL AND METHODS

As shown in Table 1 the subjects of this study were 8 newborn infants ranging in age from 10 to 127 hours at the time of the study There were 3 male and 5 female Caucasian infants Their birth weights ranged from 1 490 to 3 950 g and gestation from 34 to 41 weeks One infant s birth weight was small for date (infant number 1) otherwise all were appro-priate for gestational age None of the mothers had complications during labor and delivery All infants were jaundiced with a peak bilirubin of 10 3 to 18 4 mg/100 ml at 24 to 72 hours of age The cause of hyperbilirubinemia was Rh incompatibility in 3 ABO incompatibility in 2 prematurity in 1 scalp hematoma in 1 Three infants required exchange transfusion at 24 to 72 hours of age

For peripheral blood flow measurement venous occlusion plethysmography was used to measure calf blood flow (CBF) The method has been reported previously (7 8) and is described briefly A latex double lumen measuring cuff was applied snugly around the upper portion of the infant s left calf This measuring cuff is connected to a transducer 0–30 mmHg range (Elema-Schonander Stockholm) The signal was fed via a preamplifier into a Mingograph 81 recorder (Elema-Schonander) The volume changes within the cuff lumen is converted into pressure signal and is recorded as such on a direct writing paper The volume change in the calf tissue was induced by stop ping venous return when a subdiastolic pressure of 40 mmHg was instantaneously applied to an occluding cuff around the infant s thigh The calibration of vol ume changes in the measurement cuff was done by injecting a known volume (0 1 and 0 2 ml) of air One measurement of peripheral blood flow is the mean of 3 successive satisfactory tracings followed by a calibra tion procedure The flow is calculated on the basis of volume changes per 100 g tissue/min The errors of the method ranged from 4–6% The skin temperature of the contralateral leg ambient temperature of the incubator and temperature of the outer wall of the incubator tops were recorded by a multi-channel elec tric thermometer (Electrolaboratoriet, Copenhagen Denmark) using several thermistors placed at the ap propriate areas An isolette servo-control unit was used to control the ambient temperature of the in cubator using infant s epigastric area as the reference temperature preset at 36 0 C

When phototherapy was indicated by clinical cri teria decided upon by the house staff the infant was placed on a continuous phototherapy regime using Air

Table 1 *Pertinent data on study infants*

Infant number	Birth weight (g)	Gestation (weeks)	Cause of jaundice	Age of study (hrs)	Control calf blood flow (ml/min/100 g)
1	1 490	36	Scalp hematoma	45	7 0
				60	5 9
				72	8 7
2	2 050	34	Prematurity	70	7 4
				92	3 8
3	3 240	38	Rh incom patibility	67	6 9
				79	5 9
4	3 180	41	ABO incom patibility	77	8 8
				102	14 8
5	3 045	37	Rh incom patibility	38	14 7
				90	8 7
6	3 950	40	Unknown	10	13 5
7	3 080	39	ABO	72	6 9
				127	5 5
8	2 900	40	Rh incom patibility	80	11 8
				100	9 0
M	2 865	38		73	8 8
S E M	270	±1		±6	±0 9

Shield daylight phototherapy lamp The eyes were covered by gauze pads the infant was naked with the exception of a single diaper The illumination intensity was measured by a light meter and was found to range from 400–800 foot candle when tested at the infant's skin level

The study was performed at various times of the day and at various time intervals after the last feeding It has been shown previously that feeding could result in a significant alteration in peripheral blood flow (8) In the current study the timing of the experiment was purposely randomized to avoid the consistent effect of feeding on the calf blood flow In 4 infants phototherapy has been started prior to enrolment into the study group In these infants the phototherapy was discontinued for at least 4 hours prior to the peripheral blood flow study

During the study a control value was obtained (with 3 consecutive measurements) followed by measurements during 30 min of phototherapy and followed by 30 min of measurement while the phototherapy lamp was turned off CBF temperature measurement and respiratory rates were measured and recorded at 5 minute intervals both during the light and no light periods Sixteen studies were performed in 8 infants i e 1 infant had 1 study 6 infants had and 1 infant had 3 studies

RESULTS

As shown in Fig 1 the control calf blood flow (CBF) averaged 8 8±0 9 ml/min/100 g (*M*±S E) and increases significantly during

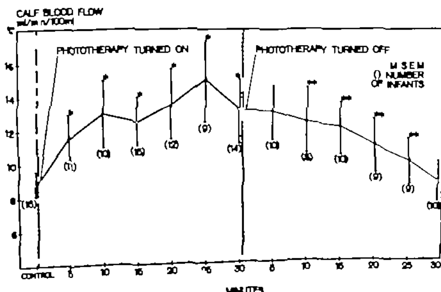


Fig 1 Changes in calf blood flow during phototherapy One asterisk indicates significant increase in values when compared with controls Double asterisk indicates significant decrease in value when compared with control ($p < 05$ by t test) Number in parentheses indicates the number of observations

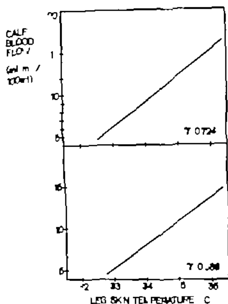


Fig 2 Correlation between calf blood flow and simultaneously measured leg skin temperature Upper graph shows values obtained at 15 min after phototherapy Lower graph shows 30 min values

the 30 min period of phototherapy The increase is at the range of 30 to 80 % above the control values The flow also decreased significantly when phototherapy was turned off All differences were significant at the level of $p <$ 05 by "paired *t* test The CBF at the end of 30 min after the phototherapy was turned off returned to the pre phototherapy level

Fig 2 shows the correlation between the calf blood flow and skin temperature simultaneously measured on the contralateral leg A direct correlation was observed with *r* values of 0 724 and 0 588 at 15 and 30 min after phototherapy respectively

The changes in the outer wall temperature of the incubator are shown in Fig 3 At 15 and 30 min after the phototherapy was turned on the outer wall of the incubator became warmer by 1 to 2 C respectively When the phototherapy was stopped the temperature fell slowly but was not statistically significant after 15 and 30 min intervals

The incubator air temperature decreased significantly 15 to 30 min after the light was turned on and increased when the light was turned off as shown in Fig 4 It should be pointed out that the incubator temperatures of the study infants were monitored by servo control units with the thermistor placed on the epigastric skin area Therefore the drop in incubator temperature may represent the compensatory mechanism of the servo control unit which shuts off the incubator warming device as the epigastric skin temperatures rises slightly at 5 to 15 min after phototherapy (from 36 1 to 36 5 C) then becoming stable during the rest of the study period (including the off phototherapy period) The rectal temperature was unchanged during the study period The respiratory rate increases signifi

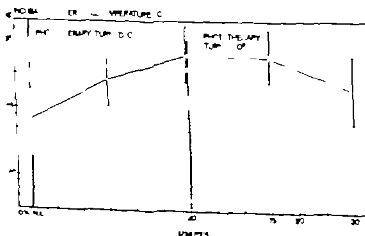


Fig 3 Change in incubator outer wall temperature following phototherapy Asterisk indicates significant increase over control values

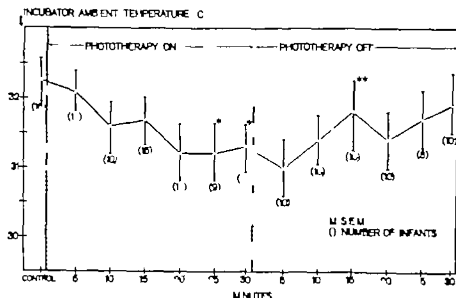


Fig 4 Incubator ambient temperature during and off phototherapy

cantly at 5 10 and 30 min after phototherapy was turned on and decreases significantly at 10 20 25 and 30 min after the light was turned off (Fig 5)

DISCUSSION

The methodology for the measurement of CBF is considered satisfactory since the reproducibility is good and the error from one determination to another is small (4–6) This method measured both skin and muscle blood flow since the flow was calculated by changes in the volume of the measurement cuff encircling a certain area of the calf tissue which included both muscle subcutaneous tissue and skin It should be pointed out that no study has been made to validate the quantitative accuracy of measuring peripheral blood flow by this plethysmographic technique However for the purpose of the present study this method was adequate since each infant served as his own control and the change in calf blood flow following phototherapy was expressed in the form of percentage change in comparison with the control values

The control values for CBF observed in the present study are consistent with those reported by others (2 4 8)

The mechanism for the increased CBF during phototherapy is difficult to assess In spite of the immediate response (within 5 min of

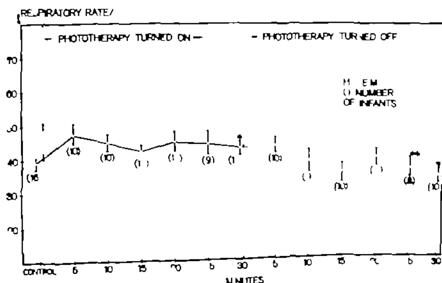


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the initiation of phototherapy) it is unlikely that the increase in flow is a result of direct local effect of radiant heat on the skin capillary. Furthermore the area where blood flow is being measured is covered by the measuring cuff which effectively shaded the area from the radiant heat. One possible reason for the increased CBF may be that the conversion of photoenergy to heat requires some means of heat elimination via increased heat loss through the skin by vasodilatation.

Within certain limits the positive correlation between peripheral blood flow and skin temperature is an accepted physiologic phenomenon (3). Our data is consistent with this concept.

The increase in respiratory rate is probably a reflection of the infant's adaptive mechanism to increase heat loss via the respiratory tracts. The increase in respiratory rate along with increased heat loss through the skin account for the increase in insensible water loss as observed previously (6). Therefore the data reported in this paper would serve as the basis for the observed increase in IWL in infants receiving phototherapy. The clinical significance is apparent. In full term infants the fluid balance may not impose a problem since they will compensate by increasing their oral intake when fed *ad lib*. However, in seriously ill low birth weight infants, particularly those receiving intravenous feeding, the increased IWL may require careful calculation and administration of parenteral fluid to avoid dehydration and its consequences.

SUMMARY

Calf blood flow (CBF), calf skin temperature, incubator wall and ambient temperature, and respiratory rate were measured in 8 newborn infants 10 to 127 hours of age who were treated by phototherapy because of jaundice. During phototherapy (within 30 min) the CBF increased to a range of 30 to 80% above the control values of 8.8±0.9 ml/min/100 g. The CBF is correlated directly with the leg skin temperature (γ=0.724 and 0.588 at 15 and 30 min after phototherapy respectively). The increase in CBF was associated with a fall in incubator ambient temperature; the latter is a result of the use of servo control unit in monitoring incubator temperature by epigastric skin temperature. The increase in CBF is probably evidence of peripheral vasodilatation to facilitate evaporative heat loss. An increase in respiratory rate was also observed during phototherapy. The observed increase in heat loss (and water loss) from vasodilatation and increased respiratory rate serve as a basis for the increase in insensible water loss as previously reported.

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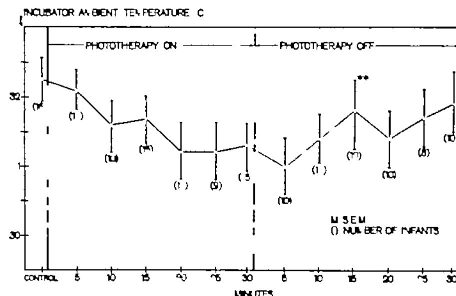


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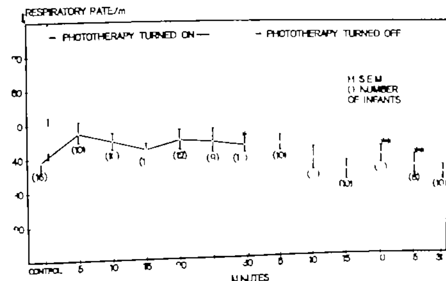


Fig 4 Incubator ambient temperature during and off phototherapy

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GENTAMICIN IN THE TREATMENT OF PURULENT MENINGITIS IN NEONATES AND INFANTS

D ZOUMBOULAKIS D ANAGNOSTAKIS A ARSENI D NICOLOPOULOS and N MATSANIOTIS

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The mortality of acute bacterial meningitis during the neonatal period and infancy despite the availability of increasingly potent antibiotics and of supportive therapy remains high and survivors frequently have crippling sequelae (2 12 13) Poor therapeutic results in infants are mainly due to failure of antibiotics generally used to control the infectious process and to eradicate the invading bacteria which commonly are Gram negative enteric organisms (7) Therefore when gentamicin became available for clinical use it seemed to merit consideration for the treatment of bacterial meningitis because of its generally high level of activity against *Escherichia coli Klebsiella enterobacter Pseudomonas aeruginosa* and *Proteus* (3)

Surprisingly although gentamicin was first isolated in 1963 very little has been published to date on experience with this drug in the newborn baby this is probably due to lack of definition of a safe and effective dosage schedule and hence fear of toxic effects on the newborn (5)

Our experience with this antibiotic in the treatment of bacterial meningitis in neonates and infants is described in this report

MATERIAL

Gentamicin was administered to 21 infants (8 neonates and 13 infants under 8 months of age) suffering from purulent meningitis who failed to improve by th "initial" antimicrobial therapy (see below)

On admission all these patients were in a very poor condition and most of them presented seizures vomiting episodes and a bulging fontanel 14 out of 21 had also hyperpyrexia

Blood cultures and complete blood counts were routinely obtained and lumbar punctures were performed on admission in all patients Cerebrospinal fluid (CSF) was immediately sent for direct cell count, smear and culture as well as for the determination of protein and glucose Subsequent lumbar punctures were performed on the 2nd the 5th the 7th the 10th day and usually 3 to 5 days prior to discharge

The initial therapy (ampicillin 300 mg/kg/24 h intravenously alone or in combination with kanamycin 15 mg/kg/24 h intramuscularly) was started immediately after blood was obtained for culture and a lumbar puncture was performed Table 1 shows the organism isolated the results of their susceptibility to antibiotics by discs method as well as some data of the patients

Five to six days after the onset of this treatment the 21 patients described here failed to improve (although the causative organism was found to be sensitive to ampicillin and/or kanamycin) and treatment with gentamicin started

Criteria for failure of the initial therapy were based on clinical grounds and findings from the CSF Most of the patients continued to have hyperpyrexia some of them still had seizures and the CFS presented more than 1 500 white blood cells per mm³ protein more than 150 mg/100 ml and glucose less than 35 mg/100 m

Gentamicin was given intramuscularly in a dose of 3 mg/kg/24 h divided into two or three portions The drug was continued for 10 12 days in all cases exept 1 patient, who received it for 20 days A complete blood count and urinalysis were done every second day and blood urea was determined once or twice a week in each patient. The state of consciousness, meningeal and focal neurological signs the temperature response head circumference and tension of the fontanel were evaluat d and recorded daily in each patient by the same observer (D Z.)

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gentamicin therapy In none of the treated infants was any toxic effect of the drug observed The blood picture remained unchanged apart from the incidence of a leucocytosis in some patients urinalyses were normal and blood urea showed only minor fluctuations

Of the 18 infants who were discharged in good condition 13 were reexamined 5–8 months later All were in good health had no neurologic sequelae or any signs of toxic after effects of the drug It should however be pointed out that audiometry tests were not performed in these 13 infants

DISCUSSION

There are several problems associated with the unsuccessful treatment of meningitis during the neonatal period and early infancy The first is late recognition of meningitis which can be a very silent infection in infancy A second problem is slow response to therapy even if it includes one or more drugs which are shown to be effective against the causative organism in vitro Finally meningitis in this age group is often caused by Gram negative bacteria such as *Escherichia coli Pseudomonas aeruginosa* and *Klebsiella enterobacter* which are resistant to most of the currently used antimicrobial drugs

The results of the present study seem to indicate that gentamicin is highly effective against bacterial meningitis in infancy Treatment was successful in 18 out of 21 cases the infection being controlled within 2–5 days

In order to place the results obtained with gentamicin in perspective it seemed pertinent to review our over all experience with purulent infantile meningitis in our department During the years 1966–1970 270 cases of infantile meningitis were treated The causative agent involved in these meningitides was a Gram negative organism in 97 cases (36%) a Gram positive organism in 35 (13%) and unknown in 138 (51%) Of the 97 cases with a Gram negative organism 54 had been caused by *Neisseria meningitidis* and 43 by another Gram negative organism The results of treatment (ampicillin in combination with kanamycin) in this latter group—which is comparable to that of the present study—were not encouraging of the 43 infants 19 (44 2) died 7 (16 3) had gross neurologic residua and only 17 (39 5) survived with no apparent sequelae (11)

It should be reemphasized that the management of the 21 patients treated with gentamicin was particularly difficult and that gentamicin was given only when the initial therapy with other antimicrobial agents had failed to improve the clinical course and the CSF findings It should also be noted that gentamicin was given only intramuscularly in no case was it given intrathecally and/or intraventricularly in this study Nevertheless our results are comparable with those of the few studies in the literature in which gentamicin was used intrathecaly and/or intraventricularly for the treatment of infantile meningitis (6 8 9 10) These latter methods of administration may produce a higher and more effective level of the drug in the CSF but it seems that the level reached with the intramuscular dose used in this study is effective against the sensitive organisms This view is supported by the findings of Klein et al (4) who treated one case with intramuscular gentamicin CSF levels were low but effective against the sensitive bacteria

In all successfully treated cases recovery was complete and permanent there were no relapses nor any evident toxic effects of the drug and the follow up of 13 babies revealed no effects on the central nervous system which might be attributed to the use of gentamicin It has been reported that various side effects of gentamicin have occurred particularly when the blood level has exceeded about 10 μg/ml (1) but this level is unlikely to be reached with usual doses if renal function is intact (8 9) Nevertheless since deafness is a very serious handicap if it occurs before the development of speech it would be wise to avoid using gentamicin and other potentially

Table 1 *The causative organism the sensitivity test and some other data from 21 infants with purulent meningitis*

No of patients	Age on admission	Causative microorganism	Sensitivity pattern[a] Highly sensitive	Moderately sensitive	Remarks
10	34 d –8 mo	*Escherichia coli*	G K	A C	
3	10 d –3 mo	*Klebsiella*	G Cp	K	One patient developed hydrocephaly
2	20 d –40 mo	*Proteus*	G	A C K	
2	15 d –2 mo	*Pseudomonas*	G Chl	A K	One baby died
2	40 d –3 mo	*Salmonella*	G A Chl		
1	14 d	*Pseudomonas*	G Chl K	A	
		and *E coli*	G K	A C	
1	12 d	*Proteus* and	G K	A C	
		E coli	G	A K C	This baby died 9 days after admission

[a] The organisms were tested by the disc method for the following antibiotics ampicillin (A) carbecillin cephalorldin (Cp) chloramphenicol (Chl) colistin (C) coxycillin gentamicin (G) betacillin kanamycin (K) novobiocin streptomycin and tetracyclines The table contains only the antibiotics to which the microorganisms were highly or moderately sensitive

Thirteen of these patients were re examined by two of us at the outpatient clinic 5-8 months after their discharge from the hospital

RESULTS

Eighteen of the 21 patients survived 2 died and 1 developed hydrocephaly The deceased patients were neonates admitted to the hospital at the age of 12 and 15 days, respectively The former a male suffering from a myelomeningocele had a mixed infection (*Proteus* and *Escherichia coli*) the latter was prematurely born and his illness almost certainly represented an exacerbation of a low-lying infection The baby who ultimately developed hydrocephaly was a 3 month-old male infected by *Klebsiella* on admission he was in a critical condition he received gentamicin for 20 days

The rapidity of recovery from meningitis was evaluated by both clinical and laboratory criteria including the state of consciousness temperature and disappearance of nuchal rigidity the concentration of glucose and protein and the leucocyte counts of the CSF

Table 2 lists the CSF findings in the 21 patients on admission 5 days after the onset of the initial treatment and 5 days after the introduction of gentamicin It can be seen that whereas a 5 day spell of initial therapy was associated with little improvement there was an almost complete recovery 5 days after the introduction of gentamicin

The mean duration of hospitalization of the 21 infants was 17 days the clinical course of the infants who ultimately recovered was already influenced 2–5 days after the onset of

Table 2 *CSF findings in 21 infants with purulent meningitis treated with gentamicin*

CSF findings	On admission Mean	Range	Five days after initial therapy Mean	Range	Five days after gentamicin administration Mean	Range
WBC/mm³	3 920	1 600–7 000	2 700	1 500–4 500	12	5–20
Protein (mg/100 ml)	570	200–960	297	150–500	62	50–90
Glucose (mg/100 ml)	24	15–35	30	15–35	43	40–50

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There are several problems associated with the unsuccessful treatment of meningitis during the neonatal period and early infancy The first is late recognition of meningitis which can be a very silent infection in infancy A second problem is slow response to therapy even if it includes one or more drugs which are shown to be effective against the causative organism in vitro Finally meningitis in this age group is often caused by Gram negative bacteria such as *Escherichia coli* *Pseudomonas aeruginosa* and *Klebsiella enterobacter* which are resistant to most of the currently used antimicrobial drugs

The results of the present study seem to indicate that gentamicin is highly effective against bacterial meningitis in infancy Treatment was successful in 18 out of 21 cases the infection being controlled within 2–5 days

In order to place the results obtained with gentamicin in perspective it seemed pertinent to review our over all experience with purulent infantile meningitis in our department During the years 1966–1970 270 cases of infantile meningitis were treated The causative agent involved in these meningitides was a Gram negative organism in 97 cases (36%) a Gram positive organism in 35 (13%) and unknown in 138 (51) Of the 97 cases with a Gram negative organism 54 had been caused by *Neisseria meningitidis* and 43 by another Gram negative organism The results of treatment (ampicillin in combination with kanamycin) in this latter group—which is comparable to that of the present study—were not encouraging of the 43 infants 19 (44 2) died 7 (16 3) had gross neurologic residua and only 17 (39 5%) survived with no apparent sequelae (11)

It should be reemphasized that the management of the 21 patients treated with gentamicin was particularly difficult and that gentamicin was given only when the initial therapy with other antimicrobial agents had failed to improve the clinical course and the CSF findings It should also be noted that gentamicin was given only intramuscularly in no case was it given intrathecally and/or intraventricularly in this study Nevertheless our results are comparable with those of the few studies in the literature in which gentamicin was used intrathecaly and/or intraventricularly for the treatment of infantile meningitis (6 8 9 10) These latter methods of administration may produce a higher and more effective level of the drug in the CSF but it seems that the level reached with the intramuscular dose used in this study is effective against the sensitive organisms This view is supported by the findings of Klein et al (4) who treated one case with intramuscular gentamicin CSF levels were low but effective against the sensitive bacteria

In all successfully treated cases recovery was complete and permanent there were no relapses nor any evident toxic effects of the drug and the follow up of 13 babies revealed no effects on the central nervous system which might be attributed to the use of gentamicin It has been reported that various side effects of gentamicin have occurred particularly when the blood level has exceeded about 10 μg/ml (1) but this level is unlikely to be reached with usual doses if renal function is intact (8 9) Nevertheless since deafness is a very serious handicap if it occurs before the development of speech it would be wise to avoid using gentamicin and other potentially

ototoxic drugs if the in vitro tests show that other less toxic antimicrobial are effective However if less toxic drugs are not effective in vitro gentamicin might be used as the agent of choice for definite therapy in some cases of purulent meningitis in neonates and infants

SUMMARY

Twenty one infants with purulent meningitis, who failed to improve after a 5–6 days parenteral administration of ampicillin, alone or in combination with kanamycin in appropriate doses were subsequently treated with gentamicin Two infants died and one developed hydrocephaly but the remaining 18 recovered and the cure seems complete and permanent Although gentamicin is an ototoxic drug it might be used for therapy in some cases of purulent meningitis in infancy when the other less toxic antimicrobial agents are not effective

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ANAL TONOMETRY

A Diagnostic Help in Hirschsprung's Disease[1]

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Hirschsprung's disease (aganglionic congenital megacolon) may usually be diagnosed clinically and by X ray. Occasionally however rectal biopsy is necessary. As the muscle itself is aganglionic this biopsy should be taken above the internal anal sphincter. More than one biopsy may be required in order to detect the aganglionic segment especially when the segment is short. Cases have also been described in which the congenital defect is localized exclusively to the internal sphincter (10). Diagnosis may be further complicated by the fact that following an initially more or less pronounced ileus or a period of constipation neonatal patients may not show clinical or radiological symptoms for periods ranging from a few days to 6 months (3 4).

Even cases with a permanent dilatation of the colon may not be Hirschsprung's disease as an idiopathic type of megacolon without aganglionosis has been described (2).

Therefore it seemed important to develop a rapid and simple method for the diagnosis of Hirschsprung's disease.

Gowers (1878) showed that a relaxation occurs in the internal anal sphincter after air insufflation into the rectum (7). This observation was subsequently confirmed by Schuster et al (11). In 1968 Tobon et al (12) described a method based upon the measurement of changes in the tonus of the internal sphincter as the normal reflex relaxation of this muscle fails to appear in patients with Hirschsprung's disease. This technique was simplified by Ustach et al. (13) and a high correlation between the results from the two methods of examination was found. Both methods were used in our study. A similar technique was used by Howard & Nixon (1968) (9) and Aaronson & Nixon (1972) (1).

[1] Read before the Danish Paediatric Society March 10 1972.

METHODS

Three balloons were used for the measurements (Fig. 1). Balloon A was placed in the rectum. B corresponded to the internal anal sphincter and C corresponded to the external anal sphincter. The balloons B and C were tied to a steel tube (Fig. 2). The pressure was measured by means of three pressure transducers and registered by means of a transducer amplifier together with a special coupling panel for adaption of the results on an ultraviolet transcriber.

In some cases the pressure was measured with an aneroid manometer (Fig. 1). Two sizes of steel tubes and two sizes of latex double balloons were used, the smaller for children under 4 years of age. After placing the balloons in the rectum air was insufflated into balloon B which was now drawn down towards the anus. Following this air was insufflated into balloon C. Balloon A was brought up through the steel tube about 3 cm cephalad to balloon B and was then inflated to the point where it just rested against the rectal wall. Pressure was measured continuously in the three balloons. The reflexes were produced by insufflation of 10 to 50 ml of air into the rectal balloon in 10 ml steps. The pressure was maintained for approximately 1 second in the rectal balloon.

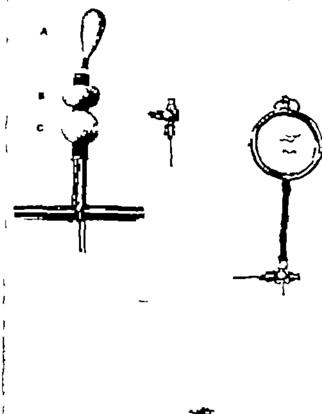


Fig 1 Large steel tube with balloons and aneroid manometer Quarter size approximately

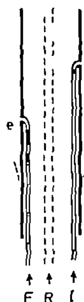


Fig 2 Cross section diagram of steel tubes The outer diameter of the large tubes is 1 2 cm of the small tube 0 7 cm The balloons are designated *E* for external *R* for rectal and *I* for internal balloon The distance between i and e is 3 0 cm for the large tube and 1 3 cm for the small tube

Table 1 *Eighteen patients with a clinical diagnosis of Hirschsprung s disease*

The patients are grouped according to treatment and the results of anal tonometry listed

Treatment before anal tonometry	No of pats	Age	Anal tonometry: Hirsch sprung response	Anal tonometry: Normal response
Operation according to Duhamel	9	3–14 yrs (22 yrs)[a]	8	1
Colon resection 15 years previously	1	41 yrs[a]	1	0
Transverse colostomy	2	9 mo 1 yr	1	1
No surgery (see text)	2	8–9 yrs	0	2
Temporary colostomy	1	4 yrs	0	1
No surgery (see text)	3	1 week–1 mo (42 yrs)[a]	3	0

[a] Three adult patients

The necessity of keeping the patient quiet during examination sometimes warranted premedication

MATERIAL

Ninety three patients between the ages of 3 days and 42 years of age were examined (Tables 1 and 2) Diazepam (0 25 to 0 5 mg/kg) was used as pre medication in 10 cases

RESULTS

Normal individuals (The internus reflex)

In all normal individuals (Table 2) typical pressure decreases were observed in the inter nal anal sphincter after air insufflation into the rectal balloon (Fig 3)

The extent and duration of the pressure decrease varied from 5–142 mmHg according to the individual patient and to the amount of air insufflated Spontaneous rhythmical con tractions with a frequency of 7 to 9 per minute were observed in the internal sphincter of 5 patients These contractions were im peded during the reflex dependent pressure decrease (Fig 4)

Table 2. *Control series 75 patients with no signs of Hirschsprung's disease*

Diagnosis	No of patients	Anal tonometry
Chronic constipation	9	Normal
Encopresis	6	Normal
Enuresis	5	Normal
Spinal cord transection (Th 7–11)	1	Normal internal sphincter, non reactive external sphincter
Anal atresia	1	Lack of sphincter function
Ulcerative colitis, colectomy and pull through operation	1	Normal
Patients without gastrointestinal symptoms	52	Normal

Hirschsprung's disease (The internus reflex)

None of the 13 patients suffering from Hirschsprung's disease (Table 1) showed reflex relaxation of the internal sphincter. One or more contractions were observed following rectal stimulation in twelve cases. The pressure increase varied from 7–222 mmHg with an average of 84 mmHg (Fig. 5). Total lack of internus reflex was observed in *one* patient.

Spontaneous rhythmical contractions with a frequency of 1 to 2 per minute (Fig. 6) were observed in 6 cases. These contractions were independent of air insufflation in the rectal balloon.

Duhamel's operation (Table I) was carried out on 9 patients where Hirschsprung's disease had been diagnosed. Postoperative anal tonometry showed an abnormal sphincter reaction in 8 patients, which was in accord with the biopsy findings. One patient, aged 2 1/2 years at the time of operation, showed normal sphincter function. Microscopy of the removed intestinal specimen showed an atypical Hirschsprung's disease without aganglionosis but with a reduction in the number of ganglion cells. Barium enema showed dilation of the entire colon.

A typical pathological pressure curve was observed in a patient aged 41, who had been operated 15 years previously.

Two patients had been treated neonatally with transverse colostomy. One showed aganglionosis and the pressure curve was abnormal while the other showed normal sphincter function. Rectal biopsy has not yet been made.

Hirschsprung's disease, to mild degree, was diagnosed in 2 patients. Exploratory laparotomy was performed shortly after birth because of ileus in the one but the only abnormality found was an elongated sigmoid colon. Now 8 years later she has normal bowel movements and normal pressure response in the sphincter. No biopsy has been

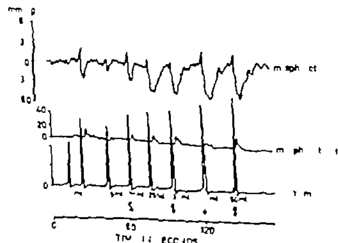


Fig. 3. Normal individual. Threshold also for the internus and externus reflexes is 15 ml air. There is no rectal reflex. The relaxation of the internal anal sphincter varies with the degree of rectal distension. (Air insufflation in the rectum is included in the registration.)

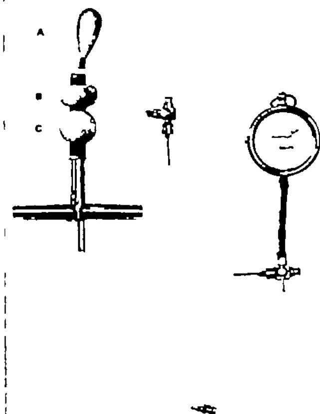


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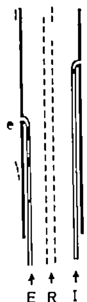


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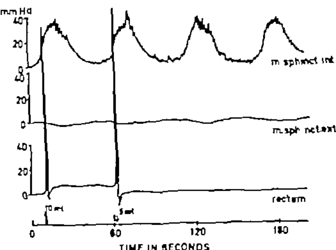


Fig 6 Spontaneous rhythmical contractions in the internal anal sphincter in a patient suffering from Hirschsprung s disease The frequency is about one per minute There are no rectosphincteric responses

Anal atresia

One patient (Table 2) who had been operated because of anal atresia did not have any sphincter function

Ulcerative colitis

One patient (Table 2) with ulcerative colitis was examined after colectomy and pull through operation The pressure conditions were on average normal but the pressure decreases in the internal sphincter were relatively small

DISCUSSION

The pathophysiology of the internal anal sphincter reflex in cases of Hirschsprung s disease is unknown It may be mentioned that the muscle is always denervated or pathologically innervated in this disease and this is connected with the fact that the development of the autonomous innervation of the intestines takes place from the neural crest in cephalad caudal direction In severe cases of the disease aganglionosis is found in both the small intestinum and in the colon although in mild cases only the rectum and the internal sphincter are involved and there are cases where the disease is exclusively localized to the internal sphincter

Possibly the abnormal sphincter reaction is due to the fact that the sphincter is only innervated by sympathetic nervous fibres An other possible explanation may be that the muscle is entirely denervated as the sympathetic connections in the aganglionic segment are broken (5 6) It is a known fact that denervated smooth musculature is abnormally sensitive to stimuli and tends to contract permanently

Although Tobon et al (12) compared patients with chronic constipation to patients with Hirschsprung s disease the control series in the present study is not homogeneous However the results may be comparable as in this study pathological sphincter reflexes were observed in all patients with Hirschsprung s disease and normal relaxation of the internal sphincter in all other patients

In order to record the rectosphincterical reflexes an electronic technique was used for the majority of patients while an aneroid manometric technique was applied in a few cases Both these methods were used in twelve cases and in agreement with Ustach et al (13) identical results were obtained As the external sphincteric reflex has no diagnostic significance a simultaneous electromyography as suggested by Tobon et al (12) was not made

With regard to threshold values and power of contraction the results obtained from other

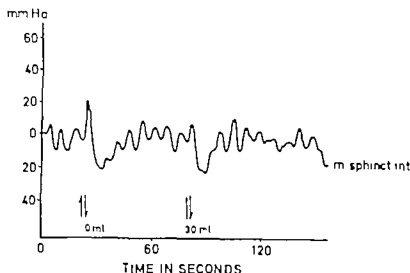


Fig 4 Spontaneous rhythmical activity in the internal anal sphincter in a normal person with a frequency of about 9 per minute The activity is impeded to some extent during the reflex pressure decrease

made The other patient 8 years old showed chronic constipation and encopresis the colon being diffusely dilated Rectal biopsy showed a reduced number of ganglion cells while anal tonometry was normal Colostomy had been performed on 1 patient due to neonatal ileus but no aganglionosis was found There were no gastrointestinal symptoms after closure of the colostomy and anal tonometry was normal

Typical Hirschsprung s disease with abnormal anal tonometry was observed in the last 3 patients in Table 1 One of them developed neonatal ileus followed by spontaneous recovery A barium enema performed later was normal while anal tonometry showed Hirschsprung s disease Three months later the patient once more developed ileus and a peroperative biopsy revealed aganglionosis

Normal individuals and Hirschsprung patients (The externus reflex)

An externus reflex was detected in 30 cases (28°) and always of the same type (10 patients were given diazepam only one had an externus reflex) An externus reflex was found in 2 cases of Hirschsprung s disease The typical externus reflex had a magnitude of 2–31 mmHg and a duration of 1–10 seconds

Normal individuals and Hirschsprung patients (The rectal reflex)

The rectal reflex (not shown) was found in 41 of all normal persons We found no rectal reflexes in cases of Hirschsprung s disease The average threshold value was 31 ml air The magnitude was 2–16 mmHg and the duration 2–20 seconds In contrast to the period of latency for the internus and externus reflexes which is less than half a second the period of latency for the rectal reflex was 3–7 seconds

Spinal cord transection

One patient (Table 2) with a spinal cord transection had a normal internus reflex but no externus reflex

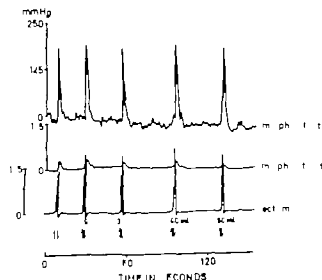


Fig 5 Curve from a patient suffering from Hirschsprung s disease On distension of the rectum the internal anal sphicter contracts i e a response inverted from the normal (Air insufflation in the rectum is included in the registration)

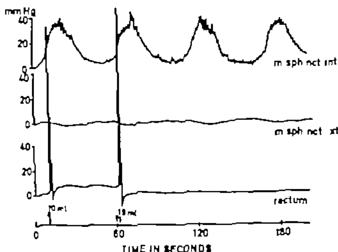


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With regard to threshold values and power of contraction the results obtained from other

studies will not be directly comparable due to varying dimensions in the measuring apparatus and to the difference in the thickness of the balloon rubber

No false positive or negative results were obtained from the present examination Tobon et al (12) and Ustach et al (13) examined 13 patients with Hirschsprung s disease and 71 without it using the technique applied in the present study and similarly no false positive or negative results were found

An absence of sphincteric reaction cor responding to the internal sphincter in prema tures during the first 14 days of life has been described by Howard & Nixon (9) Aaronson & Nixon (1) could exclude the disease in 90 8% and confirm its precence in 74 3 % The contractions shown in Fig 6 is suggested to correspond to mass-contractions without propulsive character in the aganglionic part as described by Hiatt (8)

CONCLUSION

Anal tonometry may be indicated for all cases where the diagnosis of Hirschsprung s disease is suggested In newborn with ileus where emergency operation may be necessary before biopsy can be made anal tonometry may serve as a diagnostical guide

The method is not dangerous and with the simplified technique it is easy It may be applied on ambulatory patients so that older children and adults with chronic constipation can by means of normal anal tonometry avoid investigations which necessitate hospi talization Newborn and small children should always be investigated during hospitalization

SUMMARY

Manometrical measurements of reflex re sponses in the internal anal sphincter were made in 93 patients In normal controls, a relaxation was obtained within the internal sphincter after distension of the rectum Pa tients suffering from Hirschsprung s disease showed either a contraction in the muscle or an absence of sphincteric response In 17 cases where the diagnosis of Hirschsprung s disease was established by conventional means the diagnosis was confirmed by anal tono- metry in 13 cases and disproved by this method in 4 cases The subsequent clinical course and histological findings have verified the diagnosis obtained by tonometry In one patient it was not possible to make the diagnosis by means of the usual methods anal tonometry showed Hirschsprung s disease and this was subsequently confirmed by biopsy

ACKNOWLEDGEMENTS

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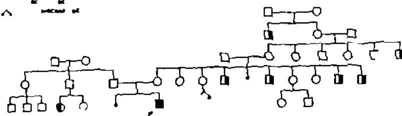


Fig. 1. Family pedigree. The proband is shown by the arrow.

LABORATORY STUDIES

With the exception of a haemoglobin of 8 g/100 ml, a strongly positive Berry spot test and a mild generalized aminoaciduria, all blood and urine routine studies were within normal limits. Radiographs of the skeleton showed osteoporotic lesions. The skull films showed slight thickening of the cranial bones. There was thickening of the base of the skull with an enlarged sella. Hand and wrist radiographs presented thickened malformed bones. The heart was enlarged and the ribs flattened on chest films. Radiographs of the spine showed sphenoidal deformity of L_1-L_4 vertebrae.

Urinary polysaccharides measured by the method of Teller et al. (5) contained 167 mg of uronic acid per gram of creatinine, which is 10 times normal for this age group (5). The polysaccharides were partially characterized as described by Kaplan (3). The molar ratio of carbazole uronic acid to hexosamine was 0.974, the carbazole to orcinol ratio 0.918 and the glucosamine to galactosamine ratio 1.606. Glucosamine is a constituent of heparan sulfate and galactosamine of dermatan sulfate. The observed ratio of these two amino-sugars suggests that the urine contained 62% heparan sulfate and 38% dermatan sulfate. This type of mixed mucopolysacchariduria with moderate excess of heparan sulfate is the pattern which is found most commonly in patients with the Hunter's syndrome (Mucopolysaccharidoses Type II) (3).



Fig. 2. The patient at 5 years of age.

DISCUSSION

The existence of "corrective factors" in the mucopolysaccharidoses was postulated because of the ability of fibroblasts obtained from normal individuals to correct *in vitro* the defect of cultured fibroblasts obtained from patients with mucopolysaccharidoses (2). Furthermore, mutual correction *in vitro* of the defect in degrading mucopolysaccharides was shown by mixing fibroblasts of patients with different types of mucopolysaccharidoses (2). It is possible, therefore, that administration of the "corrective factors" to patients might have beneficial effects. Potential approaches to the clinical management of the syndromes would accordingly be: 1) blood or plasma infusions, 2) leukocyte infusions, 3) tissue transplantation and 4) purified factor(s) infusions. Favorable reports followed the administration of fresh frozen plasma (1) and leukocytes (4) to patients affected with Hurler's and Hunter's syndromes. Administration of purified factors and tissue transplantation has not been accomplished yet.

Although both disorders affect physical appearance, it is interesting to note that the

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SHORT COMMUNICATION

HUNTER S SYNDROME AND COOLEY'S ANAEMIA IN THE SAME PATIENT

Effect of Multiple Transfusions

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Di Ferrante et al (1) recently reported beneficial effects of fresh frozen plasma infusions on patients with Hurler's and Hunter s syndromes Because of the novelty of this possible therapeutic approach evaluation has, of necessity depended upon short term observations During the last 4 years we have administered repeated transfusions to a 5 year-old patient with the Hunter syndrome who also has Cooley s anaemia The observations regarding the progress of the mucopolysaccharidosis are not as complete as they would be if this had been a prospective study designed to evaluate a new therapeutic agent However we believe that this limitation is counterbalanced at least in part by the fact that the transfusions covered a much longer time span than any other studies of this type which are now available

CASE REPORT

A 5 year old boy was admitted to the Paediatric Unit of the Aghia Sophia Children s Hospital for evaluation of mental retardation He was the product of the second pregnancy of a 30 year old mother and a 32 year old father The first pregnancy resulted in a spontaneous abortion during the third month of gestation This child was born at term with a normal delivery Splenomegaly was noticed during the second month of life

At the age of 9 months the diagnosis of homozygous beta thalassaemia was established by haemoglobin electrophoresis He was subjected to frequent whole blood transfusions initially every 2 months and recently every 15 days He received a total of 38 transfusions of whole blood 1–4 days old stored at 4 C in ACD-containing glass bottles Initial transfusions were of 150 ml blood volume each while the more recent ones ranged between 400–800 ml

Severe nasal congestion was first noticed at 3 years His psychomotor milestones were retarded At 10 months he sat without support he stood up with support at 3 years and began to walk shortly before his 5th birthday

Family history is remarkable in that a first cousin of the proband has homozygous beta thalassemia while a large number of males on the mother s side died in late childhood or adolescence with clinical features of gargoylism and mental retardation (Fig 1)

On physical examination he appeared a short pale and retarded boy His height of 97 cm was below the third percentile His head appeared big (circumference 48 cm) with coarse facial characteristics His eyes were normal The mucous membranes were red and there was constant nasal dripping The neck was short The thorax was wide with no rales or abnormal heart sounds on auscultation The abdomen was very distended with a liver palpable 12 cm below the right and a spleen 18 cm below the left costal margins (Fig 2) There was a small umbilical hernia and a scar from an inguinal hernia repair

The fingers were short and stubby There was limitation of elbow and knee joint movements He could walk a few steps with support but could not however use a spoon correctly to eat His vocabulary was limited to 4–5 words He was not toilet trained A gross estimate of his psychomotor development placed him around the 12–13 month level

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CASE REPORT

PRIMARY PULMONARY HYPERTENSION IN INFANCY

PER TEISBERG and JENS HOGNESTAD

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The term primary pulmonary hypertension (PPH) is used when no apparent cardio-pulmonary disease or condition can explain the increased pressure on the arterial side of the pulmonary circulation. Characteristic autopsy findings are hypertensive vascular changes in the lung and hypertrophy of the right side of the heart.

The condition occurs in all age groups and in both sexes, but there seems to be a predominance in women between 30 and 50 years according to the published materials (for review see Blount (1)). The occurrence of multiple cases of PPH in families has been seen (6, 9, 10, 11, 17). A few cases of PPH in infants have been reported (3, 5, 8, 11, 12, 14, 17).

This report will describe a case of PPH in a five-week-old infant with special emphasis on the pathological changes. Some etiologic and pathogenetic considerations will be included and the possible association between drug consumption and the development of pulmonary hypertension will be discussed.

CASE REPORT

The body of a five-week-old male infant was remitted to the Institute of Forensic Medicine for autopsy because of a sudden unexplained death.

The pregnancy had been uncomplicated and the boy was born at full term with a birth weight of 3 450 g and length 50 cm. Medical examination in the maternity clinic did not reveal anything abnormal, but according to the mother episodes of apparent respiratory distress and cyanosis occurred from the first days of life. Later on, when she had brought the child home, it obviously did not thrive. Episodes of vomiting with cyanotic spells were frequent, and the child failed to gain weight. No further medical examination was made until the child died 5 weeks old.

When questioned, the mother admitted that she had used amitriptyline (Saroten)® the last 3 weeks before the child was born. Similar or related cases have not been observed in the family.

AUTOPSY

The heart weight was 40 g (normal weight 25 g). There was a pronounced hypertrophy and dilatation of the right ventricular wall. The foramen ovale was open and so was a narrow ductus arteriosus Botalli. There were normal vessels and no signs of congenital anomalies in the heart. The myocardium showed pronounced hypertrophy of the individual muscle cells.

The lungs were large with a firm consistency and the air content was clearly reduced. On section the lungs showed a greyish colour with prominent blood vessels. On microscopical examination there were evenly dispersed areas of atelectasis and emphysema throughout all lobes. The alveolar walls were thicker than normal with ingrowth of connective tissue (Fig. 1). The most striking finding was a pronounced hypertrophy of the arterial tree. Both the arterial and arteriolar walls showed a thickening of the media (Fig. 2). There were no endothelial hypertrophia

patient morphologically presented only the features of a mucopolysaccharidosis Cooley's anaemia was evidenced by the splenomegaly the thickening of the skull and by the osteoporotic lesions of the skeleton such as seen on various chronic haemolytic disorders It is evident that the frequent transfusions did not prevent the development of phenotypic changes typical of the Hunter syndrome

Whatever changes the transfusions may have brought about in the mucopolysaccharidosis were insufficient to be clearly recognizable The amount of plasma contained in the transfusions was of the same order as that recommended by DiFerrante's group Our results are in no way to be considered a refutation of this possible therapeutic approach They do indicate however that any corrective factors which may be present in plasma do not withstand the customary procedures for blood storage and administration It seems clear that prospective therapeutic trials must utilize fresh or fresh frozen blood derivatives

SUMMARY

It has been reported that plasma and leukocyte infusions have a beneficial effect on patients with Hurler's and Hunter's syndromes A 5 year-old boy with Hunter's syndrome and Cooley's anaemia is presented This boy received a total of 38 transfusions over a 4 year period The usual clinical manifestations of the Hunter's syndrome were fully developed and were not, in any obvious way ameliorated by the transfusions

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SUMMARY

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and no perivascular inflammatory changes Intravascular thrombotic or embolic processes were absent There were no signs of arteritis or collagen disease in sections from heart liver spleen or kidneys

COMMENTS

Although unspecific the clinical signs as observed by the mother fit well with the description given in earlier reports on PPH in infancy

The changes observed at autopsy are characteristic of PPH of relatively short duration (3 8 11) Slight hypertrophy of the muscular media of the small and middle sized arteries is normally seen in infants 5 weeks after birth In our case upon direct comparison with lung sections from infants of this age dying from other diseases the hypertrophy was shown to be far more pronounced In cases where the pulmonary hypertension has been of long standing intimal changes can also be seen (15 18) The pronounced right heart hypertrophy and the presence of symptoms at birth indicate that the condition probably was of prenatal origin

PPH is the result of an increased resistance in the pulmonary vasculature while the heart hypertrophy is a secondary phenomenon Obstruction of the pulmonary vasculature by emboli or thromboses could induce pulmonary hypertension (15) but this mechanism need not be considered in PPH of childhood and infancy The most probable explanation is that vasospasm initially is the dominating pathogenetic component This may in the infant prevent the normal involution and initiate further hypertrophy of the media of the arteries

Animal studies indicate that serious prenatal and neonatal hypoxaemia can initiate pulmonary vasoconstriction (13) This may in some cases of PPH of infancy have served as a contributing factor but there is no evidence that this is a factor of main etiologic importance (4) Lately several reports have appeared which seem to establish an association between the use of an anorectic drug (aminorex fumarate) and the development of pulmonary hypertension in some individuals mostly young and middle aged women (2 4 7) The drug is related to amphetamine and has stimulating effects on adrenergic alpha receptors The most interesting aspect of this discovery is that a drug with sympathomimetic effects may induce pulmonary hypertension but no systemic hypertension

In our case the mother had used a tricyclic antidepressant drug amitriptyline during the last 3 weeks of the pregnancy Amitriptyline potentiates the effects of noradrenaline and adrenaline in the vessel walls and arterial hypertension may be induced (16)

It seems that the effect consists in a blockade of the catecholamine membrane pump in the granules of the post ganglionic fibres It is uncertain whether these drugs can induce hypertension when catecholamines are not supplied from exogenous sources

As far as we know there are no reports in the literature on the possible association between the use of tricyclic antidepressant drugs and the development of pulmonary hypertension Such an induction might be feasible Further clarification of the problem will have to await future reports on this rare condition The aminorex episode is a reminder that careful inquiries on drug consumption should always be made It is uncertain whether haemodynamic studies of patients using tricyclic antidepressant drugs or animal experiments could provide additional information The wide spread use of the antidepressant compared with the very few cases of PPH seen indicates that a predisposition possibly genetic would have to be postulated as a necessary additional factor This additional component may simply consist in a particular vulnerability of the fetal lung to the effect of the tricyclic antidepressant drugs

SUMMARY

A case of primary pulmonary hypertension in a 5 week-old child is described The mother

Fig 1 Section shows thickening of the alveolar septa with ingrowth of connective tissue

Fig 2 Small arteriole with proliferation of media almost causing total obliteration of the

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CASE REPORT

THREE SIBLINGS WITH ATYPICAL MUCOPOLYSACCHARIDOSIS

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After the first description of Hurler s syndrome in 1919 there were many reports describing patients with similar findings In 1957 Dorfman and Lorincz demonstrated an increased excretion of mucopolysaccharides in a patient s urine (1) and since then it has become apparent that mucopolysaccharidoses comprise several entities McKusick proposed a classification on the basis of clinical genetic radiological morphological and biochemical features (10) However it has repeatedly been shown that there are cases which defy all attempts at classification (3 5 11 14 15 16) In this report we describe three such siblings

CASE REPORTS

Case I

Y Y a 10-year-old male was found to complain chiefly of restriction of movement in his joints and being easily fatigued. According to the parents these symptoms had started 4 years before his admission Because of frequent upper respiratory infections a tonsillectomy had been performed on the patient 2 years previously He was in the fourth grade at school and a good student

The family history revealed that the parents were first degree relatives. It was also pointed out that two of his sisters were similarly affected while the brother was normal

Physical examination showed the boy s height to be 138 cm and his weight to be 30 kg (both 25th percentile) Both corneas were cloudy and his facial features were rather coarse with a prominent chin (Fig 1 *a*) There was no hepatosplenomegaly Movement of the hip joint was limited bilaterally and there was also slight scoliosis

Laboratory findings revealed Reilly granulations in practically all the neutrophils as well as metachromatic granules in about 10 of the lymphocytes X ray films revealed that the lower dorsal and upper lumbar vertebrae displayed marked retardation of ossification in their epiphyseal rings which had resulted in deep notches in the superior and inferior corners of the anterior vertebral surface and a beak like protrusion in the latter's mid portion The capital femoral epiphyses showed marked fragmentation and flattening (Fig 2) Both the femoral heads and the necks were widened and there was bilateral coxavara deformity The acetabular roofs revealed irregular ossification with marginal scalloping The metacarpal bones had a stubby appearance with relative widening of their diaphyses EKG was interpreted as normal Urinary mucopolysaccharides determined by the method of Hsia & Inouye (4) were 68 mg per liter which is higher than the normal values for our laboratory (less than 30 mg per liter)

Case II

B Y was a 7 year-old female with complaints similar to those of her brother (case I) (Fig 1 *b*) She was in the second grade and a good student Her findings were the same including cloudy corneas and in addition her height was 111 cm and her weight 18 5 kg (both below the third percentile) Her blood morphology was also similar to that of her brother X rays of the vertebrae showed no abnormalities except similar pathology in the femoral heads Again mucopolysaccharides in the urine were 50 mg per liter

Case III

P Y the younger sister who was 5 years of age had the same complaints (Fig 1 *c*) Interestingly though, her height was 104 cm and her weight 17 5 kg which falls close to the 10th percentile Again there was no hepatosplenomegaly

Laboratory examination of this child showed the

had used amitriptyline in the gestational period and the possible induction of pulmonary hypertension by this drug is discussed

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Table 1 *A classification of Mucopolysaccharidoses*

Eponym and type	Corneal clouding	Claw hands	Dwarfism	Mental retardation	Bony changes	Reilly Gr	Urinary MCP
Hurler I	+	+	Marked	Marked	Severe	− +	HS–DS
Hunter II	−	+	Marked	Mild moderate	Severe	− +	HS–DS
Sanfilippo III	−	+	Moderate	Marked	Minor	− +	HS
Morquio IV	Late	− +	Marked	—	Severe	− +	KS
Scheie V	+	+	Mild	—	Moderate	−	DS
Maroteaux Lamy VI	+	+	Marked	—	Severe	+	DS
Present cases	+	−	None	—	Severe	+	HS–DS

Key to symbols MCP Mucopolysaccharides HS Heparan sulfate DS Dermatan sulfate KS Keratosulfate

sulfate and heparan sulfate in the urine characteristic bony changes and corneal clouding our cases may be considered to be examples of Hurler s syndrome but the absence of mental and growth retardation makes it difficult to classify them definitely as such At the same time the absence of the claw hand the presence of quite severe osseous changes as well as the Reilly granulations in the leucocytes differentiate the patients from those with Scheie s syndrome Osseous changes especially fragmentation on the proximal epiphysis of the femur Reilly granulations and the absence of mental retardation are features commonly seen in the Maroteaux Lamy syndrome (8 11) *Although* the excretion of small amounts of heparan sulfate does not necessarily exclude the diagnosis of Maroteaux Lamy s syndrome (2 12) the presence of appreciable amounts of heparan sulfate in the urine and the normal growth pattern observed in these cases makes *this* diagnosis rather unlikely

In summary we feel that these patients are atypical variants and are difficult to categorize It is interesting to note however that a hypothesis has been put forward by Mc Kusick et al which seems to shed light on this subject (13) On the basis of cellular studies they proposed that genes for certain mucopolysaccharidoses are allelic and the intermediate forms or atypical variants are due to a genetic compound of these genes or to a homozygosity of a new allele at the same locus (13)

In conclusion it is clear that a classification cannot be assured with a significant degree of confidence until specific enzyme deficiencies for the different mucopolysaccharidoses are discovered We record here with satisfaction that significant advances have already been made in this respect as recent publications indicate (9 13)

SUMMARY

Three siblings with mucopolysaccharidoses are presented They were found to excrete dermatan sulfate and heparan sulfate and had specific bony changes without any physical or mental retardation These features were compared with those of other known cases of mucopolysaccharidoses

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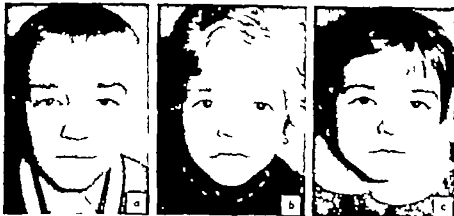


Fig 1 Facial appearances of the patients (*a*) Case I (*b*) Case II (*c*) Case III

same findings as in the first case including the beaking of vertebral bodies (Fig 3) and femoral epiphyseal changes Mucopolysaccharides in the urine were 70 mg per liter

Chromatographic separation of urinary mucopolysaccharides in these patients (8) showed about 65 dermatan sulfate and 35 heparan sulfate

DISCUSSION

On the basis of clinical radiological genetic morphological and biochemical criteria six distinct mucopolysaccharidoses have been differentiated (10 11) These are types I II III IV V and VI or eponymically the Hurler Hunter Sanfilippo Morquio Scheie and Maroteaux Lamy syndromes All the patients were found to excrete either heparan sulfate dermatan sulfate or both in the urine in excessive quantities (Table 1) Although some authors have suggested that mucopolysaccharidoses may be classified by the type of mucopolysaccharide found in the urine (6) others do not believe that the urinary excretion pattern alone supports classification (7 12)

On the basis of the excretion of dermatan



Fig 2 X ray of the hip in Case I showing fragmentation of the capital femoral epiphyses widening of the femoral heads and necks and bilateral coxa vara



Fig 3 X ray of the spine in Case III showing deep notches at the corners and beak like protrusions along the mid portion of the anterior surface of the vertebrae

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CASE REPORT

BILATERAL TIBIAL APLASIA WITH LOBSTER-CLAW HANDS

A Rare Genetic Entity

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The familial type of congenital tibial aplasia associated with split hand is a very rare anomaly (1 2) It is usually regarded as being caused by a single dominant gene with low penetrance and variable expressivity We present here the sixth report of this specific familial entity in the literature

Our patient (V 1 on pedigree Fig 1) was born after a full term uneventful pregnancy Examined at the age of 15 months (Fig 2) he was found to have a lobster-claw deformity of the right hand with absent middle finger Both thighs were held in the frog position. There was increased external rotation (90) and diminished internal rotation (10) of both hips The knees had a 90 flexion contracture There was a marked equino-varus deformity of the feet In addition the right foot had four normal toes and a rudimentary big toe Radiographic examinations revealed bilateral absence of the tibiae hypoplasia of the right ilium absence of ossification centre of the right capital femoral epiphysis The right foot had absent ossification centre of the talus as well as absent 1st metatarsal There was considerable delay in the ossification centres on the right as compared with those on the left side The chromosome karyotype was normal

The parents of our patient are of Palestinian moslem origin and are consanguinous However since consanguinous marriages are relatively common among Palestinian moslems we do not attach great importance to this fact in

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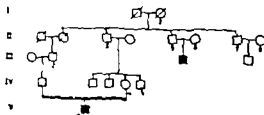




Fig 2 General view of malformations of the extremities.

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Key words Mucopolysaccharidosis, dermatan sulfate heparan sulfate

CASE REPORT

GOLDENHAR S SYNDROME

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Goldenhar s syndrome or oculo-auriculovertebral dysplasia is a rare malformation syndrome consisting of *ocular* manifestations (epibulbar dermoid or coloboma of the superior lid) *aural* manifestations (pre auricular tags or fistulae or more severe ear malformations) *vertebral* malformations (fused cervical vertebrae hemivertebrae supernumerary thoracic or lumbar vertebrae spina bifida) (1 2 3 4) A newborn girl with the typical syndrome (conjunctival epibulbar dermoid buccal cleft with micrognathia pre auricular tags and fistula multiple hemivertebrae 13 ribs) (Fig 1) had as unusual features mucosal tags along the lateral border of the tongue and an extra thumb (Fig 2) Normal chromosomes no heredity known normal parents and one older sister



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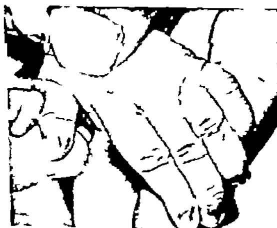


Fig 2

interpreting the pattern of inheritance More over a relative of the patient (IV 3) is reported to have identical anomalies but without parental consanguinity Based on our pedigree we favour the single gene autosomal dominant pattern of inheritance with reduced penetrance

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LETTER TO THE EDITOR

Sir

Kildeberg in his review of my book *Clinical Acid–Base and Electrolyte Balance* has mainly dealt with arguments against my use of Base deficit$_{ECF}$. His criticism is surprising because the theoretical and experimental background for this is demonstrated (Siggard Andersen Rooth & Jacobson). The advantage of using a Base deficit which is unaffected by an increase in P_{CO_2} is obvious and on this point his criticism cannot be accepted. In pure metabolic disturbances and such cases are rare as there is usually a concomitant compensatory change in P_{CO_2}. Base deficit$_{ECF}$ is perhaps superfluous but not theoretically wrong. It is just as correct to describe a mean Base deficit of the extracellular fluid as to describe the Base deficit of the circulating blood.

For the clinician it is unnecessarily complicated to use different Base deficit values and BD_{ECF} appears to be the best choice. For physiological studies BD_i and BD_p is often needed but to press upon the clinician the complicated interactions between the red cells the plasma the interstitial and intracellular fluids will not help him in dealing with the patients.

Gösta Rooth

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Sir

Thank you for inviting my comments on the letter by Gösta Rooth.

The relatively short history of clinical acid base chemistry is largely one of gallant attempts to identify "respiratory" and "metabolic" sources of change in the acid–base status of blood by direct measurement (with the foreseeable result of a set of operational definitions)—and in the face of failure by less gallant twisting of the primary data. Whereas clinicians since long have given up the easy notions that a high body weight proves presence of surplus body fat or that a low concentration of calcium in serum proves loss of calcium from the body, the trust in the obscure magics of acid–base analysis and in the ingenuity of its pursuers seems inextinguishable.

From a physiological point of view there are three qualitative categories of acid and base (carbonic acid, metabolizable organic acid and base, and non-metabolizable acid and base); there are three quantitative variables of their protolytic effect (pH, buffer value and titratable acidity—of which two are independent) and there are three ways in which a change in the titratable contribution by any of the above categories can come about (change in balance, change in solvent volume and change in distribution). Clearly the number of diagnostic permutations exceed the conventional clinical vocabulary (metabolic and respiratory acidosis and alkalosis). The only sensible approach is the application of *physiological interpretation* (by the clinician) to *primary chemical data* (by the clinical chemist). Neither the CO_2 machine nor the "Astrup machine" has ever established a clinical diagnosis—let alone cured a patient.

The arguments presented in my brief review of Rooth's publication need no repetition. Rooth pinpoints his position by writing: "The advantage of using a Base Deficit which is un

LETTER TO THE EDITOR

Sir

We read Growing Pains—A Clinical Investigation of a School Population (1) with interest Øster & Nielsen have in a school age population outlined the incidence of a pediatric condition so common as to be usually overlooked

We reported a similar study from an ambulatory pediatric population (2) Further we outlined the patterns of pain for each muscle *group involved and for each found a small* hypersensitive area which Travell & Rinzler (3 4) termed the trigger area Coolant spray can be applied to the trigger area with a high degree of success in relief of the associated pain pattern

Some children with growing pains —or myofascial pain in childhood —as we termed it—are handicapped by their pain and the ability to outline the pain pattern and relieve *it with coolant spray is* of importance to the physician Technique of use of coolant spray and of the occasionally necessary adjuvant injection therapy has been described (2) and the pain patterns and associated trigger areas illustrated

The coming generation of physicians reading Øster & Nielsen s demographic study (and the authors state that a paper is planned for Pediatrics as well) would be well reminded that this condition can be successfully treated as well as diagnosed

Talcott Bates *Edgar Grunwaldt*

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PAEDIATRIC ENDOCRINOLOGY

Meeting in Louvain September 7–10 1972

I Z Beitins F Bayard I G Ances A Kowarski & C J Migeon (Baltimore) *Transplacental passage of glucocorticoids in pregnancy near term*

A constant infusion of ^{14}C cortisol and ^{3}H cortisone for 4 hours was given to pregnant women at term at the time of elective cesarean section Radioactive and nonradioactive cortisol (F) and cortisone (E) concentrations were determined in maternal and cord plasma at the time when the concentration of the radioactive steroids had reached a plateau Metabolic clearance rates (MCR) plasma levels of endogenous F and E blood production rates (BPR) conversion ratios (CR) and transfer constants ($[\rho]_{BB}$ values) were calculated and compared with those obtained in non pregnant women half of them taking contraceptive medication

The MCR (F) of women near term normal women and women receiving contraceptive treatment were (mean ± S D) *133 ± 47 141 ± 37* and *62 ± 24 l/24 hrs respectively The latter* was significantly lower than the other two The MCR (E) were *similar* in all three groups of subjects and 4 to 7 times greater than MCR (F)

The ratio of the endogenous F/E in normal women was significantly lower (4 8 ± 0 6) than that of women on the pill (9 0 ± 1 6) or of pregnant women (7 5 ± 1 7) In contrast to their mothers the newborns had an F/E ratio of 0 85 ~ 0 34 with a mother cord ratio for F of 4 9 ± 2 5 and for E of 0 50 ± 0 15

In 8 fetuses of 3 to 6 months of gestational age plasma concentrations of F (2 1 ± 1 2 μg/100 ml) and E (4 7 ± 3 3 μg/100 ml) were lower than those of 6 newborns (6 3 ± 2 9 for F and 7 2 ± 1 2 μg/100 ml for E)

From the values of BR (F) and the transfer constant $[\rho]_{BB}^{F\to E}$ it was estimated that all the BP (E) of the subjects studied arose from BP (F) and therefore there was no E secretion

In previous studies in sheep it was demonstrated that cortisol crossed the placenta from the mother to fetus but that the fetus near term was also able to secrete cortisol In pregnant women we were also able to calculate the maternal contribution to the fetal cortisol and cortisone concentration and to show that the fetus secretes three fourths of its cortisol but that its cortisone was mainly maternal in origin

The transplacental passage of ^{3}H prednisone and ^{3}H prednisolone was also studied It was found that irrespective of whether ^{3}H prednisone or ^{3}H prednisolone was infused the concentration of ^{3}H prednisolone in the maternal plasma was significantly higher than that of ^{3}H prednisone The maternal ^{3}H prednisone concentration was similar to the fetal ^{3}H prednisone concentration but the maternal ^{3}H prednisolone concentration was 8 to 10 times greater than the fetal ^{3}H prednisolone Pregnancy and contraceptive medication had no effect on the MCR of prednisone or the conversion ratio of prednisone to prednisolone The MCR of prednisolone was significantly lower than that of prednisone in both pregnant and non pregnant women

affected by an increase in P_{CO} is obvious
In my view the *dis*advantage of such usage is obvious—for the very simple reason that changes in P_{CO} inevitably lead to changes in the concentration of base in blood There are no reasons why such changes should not exist and Rooth will have to live with them Perhaps unknowingly he has already made a start On page 9 of his book the word primary appears in his definitions of metabolic acid-base disturbances[1]

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the newborn pituitary and this stimulus is responsible for the state of physiological hyperthyroidism which characterizes the neonatal period Available data suggests that this thyrotropin response occurs as a result of skin cooling which inevitably occurs as the fetus is delivered into the extra uterine environment

G Hers (Louvain) *Recent developments in the biochemistry of hormone action*

M G Forest A M Cathiard & J Bertrand (Lyon) *Plasma testosterone analysis by radioimmunoassay Normal values in newborn infants and prepubertal children*

Plasma testosterone was measured by radioimmunoassay using antirabbit antibodies Testosterone carboxyoxime was coupled to BSA in the 3 position A titre of 120 000 of antisera 6 II a was obtained after 7 weeks of immunization Among 20 steroids tested only dihydrotestosterone (DHT) had a significant cross reaction with testosterone 1 000 cpm of H^3 testosterone were added to alkalinized plasma which was extracted with ethyl ether Disposable celite columns allowed an excellent purification of testosterone from DHT DHA Δ^4 androstenedione estradiol estriol and C^{21} steroids Iso-octane was used as carrier solvent and the 25% ethyl acetate in iso-octane fraction eluted testosterone The dry extract was dissolved in 1 ml PO_4 buffer (0 1 M pH 7) 0 2 ml was counted for recovery and three aliquots (0 1 0 2 0 4 ml) assayed on radioimmunoassay Equilibration was performed at 4 C overnight Free and bound fractions were separated using Dextran (0 05 %) Charcoal (0 5) in PO buffer A standard curve (0 to 1 000 pg) was run in duplicate simultaneously The method gave a blank of 13 pg (n = 86) Intra and inter assay variation coefficients were similar (3 76%)

Testosterone values in normal adults were 565 ± 146 and 37 2 ± 9 6 ng/100 ml in males and females respectively In 62 children similar values were found in both girls and boys (6 58 ± 2 48 and 6 62 ± 2 46 respectively) 25 normal boys gave a response to HCG stimulation of 656 ± 207 ng/100 ml There was no difference in testosterone basal levels in prepubertal children of both sexes 1 to 10 years of age In contrast a wide range of testosterone was found in 21 newborn studied with higher concentrations in the male infants 3 to 8 days of age (38 5 ± 26 7 and 13 8 ± 7 2 in male and female infants respectively)

W M Teller B Rane & S B Pal (Ulm) *The excretion of Δ^5 pregnenetriol in children of various ages*

Dehydroepiandrosterone (DHA) excretion in the urine is very low or absent during early childhood except for the neonatal period It rises gradually towards the onset of puberty Δ^5 Pregnenetriol (pregn 5-ene 3β 17α 20α triol) (Δ^5 PT) has the same precursor as DHA Therefore the urinary excretion of Δ^5PT during childhood was studied to see whether it follows the same pattern as DHA Twenty four hour urines were collected from 55 children without endocrinopathies I 12 newborns aged 0–4 wks II 12 infants aged 1–12 mo III 9 small children aged 1–6 yrs IV 8 school children aged 7–10 yrs V 7 preadolescents aged 10–12 yrs VI 7 adolescents aged 12–15 yrs The method of determination of Δ^5PT (Pal & James 1964) included hot hydrolysis for two hours at pH 7 filtration and extraction with ether ethyl acetate (2 1 v/v) fractionation by Girard reagent T into ketonic and non ketonic portions oxidation of the non ketonic fraction with metaperiodate to form DHA paper chromatographic purification final determination of the newly formed DHA by Zimmermann reaction Simultaneously DHA was determined in the ketonic fraction Recovery of total procedure was checked by tritiated DHA sulphate added to samples prior to hydrolysis and amounted to 87% The results obtained were as follows (mean excretion per 24 h with range) I Δ^5PT 0 07 (0 01–0 11) DHA 0 07 (0 04–0 12) II

J M Saez & A M Morera (Lyon) *Plasma oestrogens before puberty in human*

It is generally accepted that urinary and plasma androgens increase during the 2 years pre ceding the onset of puberty in human partic ularly when clinical manifestations of what L Wilkins called Adrenarche are present

Oestrogen participation has not been fully studied as yet in spite of the fact that urocytograms or some biological assays were in favor of an increase of their production during pre puberty Urinary excretion of Oestrone (E_1) Oestradiol (E_2) Oestriol (E_3) has been docu mented during this period however these re sults were obtained with techniques of low specificity and were not correlated with plasma values

Plasma E_1 and E have been assayed by means of a radioimmunological technique be fore puberty in normal children and in chil dren with different pathological conditions Results clearly show that in both sexes, there is an increase of E_1 production by the adrenal during the pre pubertal period

In both girls and boys 1 to 6 years of age plasma values for E_1 and E range from 0 3 to 1 ng per 100 ml In older children of both sexes 7 to 10 years of age there is a marked increase of E_1 *concentration while only a slight but not* significant increase in E concentration is noted The average value for E_1 was then 3 6 ng/100 ml

In girls with precocious puberty there is a *predominant* increase of E whereas in children with premature pubarche plasma values of E_1 predominate over E

In cases of Turner syndrome 6 to 12 years of age plasma E_1 concentration was compar able to normals of the same age but E values were very low

The adrenal origin of E_1 can be demon strated by means of its Dexamethasone sup pression in 4 girls with precocious puberty whereas in these cases fluoxymesterone admini stration can achieve a comparable decrease *in* plasma E only Moreover this has been even more evidenced in adults during adrenal vein catheterisation

D A Fisher (Torrance California) *Thyroid function in the fetus & newborn*

The human fetal thyroid is capable of con centrating iodine and synthesizing iodothyronines by 12 weeks gestation And TSH is present in the fetal pituitary and in fetal blood by that time However studies of total and free thyroxine (FT 4) and TSH concentrations in fetal blood have shown very low levels prior to 18–20 weeks suggesting that hypothalamic TRH secretion is low at that time Between 18 and 22 weeks there is a rather abrupt increase *in fetal pituitary* and serum TSH concen trations followed by a progressive increase in fetal serum total and free thyroxine concen trations FT4 levels may actually exceed the maternal values at term This data and data of Evans et al indicating a significant increase in fetal thyroidal radioiodine uptake between 17 and 21 weeks suggest that the fetal hypo thalamus functionally matures between 15 and 20 weeks

Studies of fetal thyroid hormone turnover during the *last trimester of gestation in the* sheep have shown very high values on a body weight basis

Total fetal thyronine secretion in the fetus exceeds the maternal value 7 times And in terestingly the maternal T4/T3 turnover ratio is 3 8 whereas the fetal value is 28

Studies of placental transfer of thyronines using dual label experiments show no transfer of T4 and only minimal transfer of T3 These data document a state of relative fetal thy roidal hyperactivity during the last trimester of pregnancy and indicate that the fetal pituitary thyroid system is functioning auto nomously of the maternal axis Moreover the fetus must have a high T4/T3 secretion ratio and/or a reduced rate of peripheral T4 to T3 conversion

Finally in the early minutes after birth there is a marked acute release of thyrotropin from

plasma deoxycortisol level in the short metyrapone test and by the largest daily excretion of 17 ketogenic steroids in the 5-day metyrapone test Plasma cortisol was determined by two-point fluorometry and deoxycortisol by a competitive protein binding method after carbon tetrachloride extraction

Following the single oral metyrapone dose plasma cortisol concentration had decreased to a very low level in one hour and this was maintained for two hours at least Plasma deoxycortisol concentration increased linearly during this 3 hour period

The precision of the tests wa assessed by the coefficient of correlation between the second and first result of the test in the individuals For the corticotrophin test the coefficient was $r = 0.87$ when both tests were performed separately ($n = 22$) $r = 0.83$ when the first test was separate and the second post insulin ($n = 31$) and $r = 0.83$ when both tests were post insulin ($n = 35$) Thus the corticotrophin test may routinely be performed in connection with insulin test The coefficient was $r = 0.76$ for the insulin test ($n = 117$) $r = 0.75$ for the vasopressin test (n 37) and $r = 0.80$ for the 3-hour metyrapone test ($n = 22$)

The 3 hour metyrapone test gave a differentiation between normal and corticotrophin deficient children which was very similar to the differentiation obtained with the insulin test Several children who were normal by the vasopressin test were corticotrophin deficient by the 3-hour metyrapone test These children presumably had a hypothalamic lesion Several children who were co ticotrophin-deficient by the 5-day metyrapone test were normal by the 3 hour test Thus the 3-hour metyrapone test was found to be more accurate than the conventional long test and equal to insulin test in asses ne integrity of corticotrophin secretion

Insulin test response was lesser than the corticotrophin test response in normal children While both responses were decreased the relative difference between them was larger in corticotrophin-deficient children In contrast the responses were almost identical in children with primary adrenocortical disease or prolonged glucocorticoid medication Similar comparison of responses to the 3 hour metyrapone test and the corticotrophin test gave an even better differentiation between primary and secondary adrenocortical deficiency

P Saenger D Shanies & M I New (New York) *Inhibition of testosterone metabolism by cultured human fibroblasts A model for drug steroid interaction*

An in vitro model for drug steroid interaction was designed utilizing cultured human skin fibroblasts Using this system an investigation of the inhibition or stimulation of enzymatic metabolism of testosterone by medroxyprogesteroneacetate (MPA) was undertaken It had been previously shown (Shanies 1972) that cultured fibroblasts actively metabolize testosterone either via the 17β hydroxyl pathway or by the 17 ketonic pathway The former resulted in the production of dihydrotestosterone (DHT) and 3α androstanediol (A diol) while the latter resulted in Δ4 androstenedione (Δ4) androstanedione (A dione) and androsterone (A) as metabolites Cells from prepubertal children of either sex utilized this 17β hydroxyl pathway predominantly while cells from adults utilized this pathway to a much smaller degree

Individual cell lines from prepubertal children were incubated with ^{14}C testosterone alone or with ^{14}C testosterone ($\sim 1 \times 10^{-7}M$) plus MPA ($6.5 \times 10^{-6}M$) Each metabolite of testosterone and unchanged testosterone were purified to constant specific activity The metabolism of testosterone by cells incubated with MPA was significantly different from controls although testosterone utilization was unchanged MPA treated prepubertal cells continued to use the 17β hydroxyl pathway as the major pathway for testosterone metabolism However there was a marked reduction in the formation of A diol at 48 hrs incubation time DHT was generally unchanged while in some experiments it was increased Since DHT is

Δ^5PT 0 1 (0 02–0 19) DHA 0 5 (0 01–0 1) III Δ PT 0 04 (0 01–0 07) DHA 0 05 (0 03–0 09) IV Δ PT 0 13 (0 02–0 25) DHA 0 11 (0 04–0 16) V Δ^5PT 0 13 (0 05–0 28) DHA 0 13 (0 04–0 24) VI Δ^5PT 0 28 (0 11–0 55) DHA 0 16 (0 08–0 21) During childhood there is a close correlation between the urinary excretion of DHA and Δ^5PT

F Bidlingmaier & D Knorr (Munich) *Plasma estrogens in childhood and adolescence*

Plasma estrone (E_1) and estradiol (E) were determined by radioimmunoassay Estrogen antibodies were produced in rabbits by immunizing with estradiol 17β hemisuccinate linked covalently to bovine serum albumin The 1 4 000 diluted antiserum bound estrone and estradiol to the same high degree There was no cross reaction to non phenolic steroids within physiological ranges but a significant cross reaction with estriol After ether extraction of 1 to 5 ml plasma the estrogens were purified by the method of Mikhail et al using Sephadex LH 20 The separated estrone and estradiol fractions were subjected to radio immunoassay

The mean reagent blanks of our method were 2 5 pg and the sensitivity was 5 pg per sample The interassay coefficiant of variation —determined on pooled children s plasma—was 12 for estrone and 10 6 for estradiol

Normal ranges were determined by investigation of cord blood (15 samples) newborns (15) prepubertal and pubertal males (52) and females (49) and young men (25) and women (25)

In cord blood both estrone and estradiol averaged 15 000 pg/ml Blood drawn from newborns shortly after birth showed levels between 300 and 500 pg/ml These levels fall to 5 to 15 pg/ml within a few days This is the normal range in both sexes up to 8 years Thereafter and in early puberty the average values in boys raise to the values of adult men (E_1 = 32 6 ± 8 pg/ml, E = 22 5 ± 5 pg/ml)

In girls the increase of estrogens after the eighth year and during puberty exceeds that of pubertal boys With menarche plasma estrogens reach levels of women with a wide variation dependent on the cyclus (E_1 = 15–120 E = 15–180 pg/ml) In 23 pubertal boys with gynecomastia no higher levels were found compared with normal boys in the same stage of puberty Most of the 20 boys and girls with congenital adrenal hyperplasia had abnormal high estrogens Highest values significantly correlated with plasma 17 hydroxyprogesterone were found in untreated or insufficiently treated patients

Ten males and 5 females with hypopituitarism aged 10 to 19 years had the expected abnormal low levels corresponding to their infantile status 5 boys with anorchia showed very low estrogens estradiol was almost unmeasurable Abnormal low levels were also found in 5 females with Turner s syndrome and XO karyotype 2 girls with XO/XX mosaic and XX (p-) karyotype had normal estrogens Clinically both showed signs of advanced puberty

S Leisti & J Perheentupa (Helsinki) *The precision and correlation of hypothalamic pituitary adrenocortical tests in children*

Corticotrophin test (0 25 mg of 1–24 peptide per 1 73 m i v) insulin test (4 U per m i v) vasopressin test (5–10 IU of lysine vasopressin i m) a new three hour metyrapone test (1 0 g per m orally at 8 a m) and the regular 5 day metyrapone test (250–500 mg orally every 4 hours for 12 doses) were performed in all or some of 117 children being assessed for the integrity of the hypothalamic pituitary adreno cortical axis The four first mentioned were repeated in some to determine the precision of these tests Corticotrophin test was performed either separately or 2 h after the injection of insulin The responses were evaluated by the highest plasma cortisol level obtained in the insulin and the vasopressin test by the two hour plasma cortisol level in the corticotrophin test by the three hour

H Gleispach & J Glatzl (Innsbruck) *Steroid pattern excreted in the congenital adrenal hyperplasia*

K E Petersen I Tygstrup & E Thamdrup (Copenhagen) *Isolated hypoaldosteronism—anatomical or biochemical defect*

We have earlier presented a boy with severe salt loss due to isolated hypoaldosteronism (resembling the cases first described by Visser et al) The aldosterone secretion was very low and remained low on sodium restriction—in spite of adequate stimulation from the renin angiotensin system as measured by renin in plasma The cortisol secretion was in the low normal range and secretion of corticosterone high

According to the findings the child was treated with sodium chloride and DOCA but he did not receive glucocorticoids He survived until the age of 18 months At the post mortem blocks of fat were found mimicking tissue located at the upper renal poles At histological examination it was impossible to find organised (adult) adrenal cortex

One year before the birth of our patient the parents lost another boy 5 weeks old Searching back it was possible to find and demonstrate the same pathological changes in this boy

The small lobules of cells found were impossible to distinguish from fetal adrenal cortex The picture does not seem to be like that in Vissers patient but seems like the so-called cytomegalic type of congenital adrenal hypoplasia

The testes in our patient were advanced in maturation—as judged from the ductus diameter the lumination the number of spermatogonia and the finding of precursors of Leydig cells The 17 keto-steroid excretion was normal during the patient's life but plasma testosterone high on two occasions

N Josso (Paris) *Inhibiting activity of the bovine foetal testis on the rat foetal mullerian duct in vitro Comparative activity of seminiferous tubules and interstitial tissue*

Though it has long been known that the mullerian inhibiting hormone secreted by the foetal testis is not an androgen no information is available concerning the nature of the testicular cells which produce this substance Having demonstrated earlier the interspecific character of the mullerian inhibiting substance we have studied the effect of various components of calf foetal testicular tissue on the foetal rat mullerian duct in vitro Testicular tissue was collected from 17 foetal calves at a local slaughterhouse Two foetuses were over 200 days post insemination and the others under 160 days according to crown–rump length Seminiferous tubules and interstitial tissue were separated according to a modification of the method of Christensen & Mason and maintained in organ culture Mullerian inhibiting activity was assessed *in vitro* using the mullerian duct of the 14 $^1/_2$-day-old foetal rat as target-organ Neither component of testicular tissue from the two older calf foetuses exhibited any mullerian inhibiting activity In contrast in the 15 younger foetuses whole testicular tissue inhibited the mullerian duct in 37 cases out of 38 and isolated seminiferous tubules did so in 22 cases out of 25 Isolated interstitial tissue inhibited the mullerian duct of only 3 tracts out of 18 and then contamination with tubular fragments was evident It is concluded that the mullerian inhibiting activity of the foetal bovine testis derives mainly from the seminiferous tubules

A Prader M Zachmann G Murset A Ferrandez & M Bambach (Zurich) *Treatment of excessively tall stature in children (girls and boys) with sex hormones The effect of estrogens and testosterone on growth and skeletal maturation*

In some tall children whose predicted mature height is excessive there is an urgent psychosocial indication and sometimes an organic one (progressive kyphosis) for treatment de

the precursor of A diol and requires 3α dehydrogenase enzyme for metabolism to A diol the data suggest an inhibition of the enzyme 3α dehydrogenase by MPA No new polar or nonpolar metabolites were detected

These experiments have shown that MPA in vitro can alter the cellular metabolism of testosterone MPA is used clinically in the treatment of precocious puberty and has been implicated as a cause of virilism in the female fetus Effects of MPA on steroid metabolism may be studied with this model system and may elucidate the mechanism of action of MPA The cell culture system described may also be used as a model to study other drug steroid interactions

A M Bongiovanni (Philadelphia) *Serum steroids in adrenal hyperplasia due to 21 hydroxylase deficiency*

Methods have been developed for the measurement of 17 hydroxyprogesterone (17 HP) pregnanetriol (PT) and 17 hydroxypregnanolone (17 OHP) in serum by gas chromatography The serum was first extracted for 17 HP directly with ethyl ether This portion was then partitioned between 70 % ethanol and petroleum ether Following derivitization this was applied directly to gas The serum was then hydrolysed with mammalian glucuronidase The PT and 17 OHP was then extracted with methylene chloride It was necessary to submit this extract to preliminary column chromatography prior to gas In all instances the trimethyl silyl esters were formed of the methoxime derivatives for application to gas Chromatography was on QF 1 at 202 C In several normals of various ages none of these three steroids was found in serum The 17 HP was elevated in all cases of 21 hydroxylase deficiency being higher in the older patients In the newborn infant the values varied between 0 17–0 48 μg% but the pregnanetriol was in all instances much higher varying between 11–15 μg In older subjects the values were 38–45 μg% for 17 HP and 90–200 μg% for PT It is notable that pregnanetriol always exceeds considerably the levels of 17 HP And also that these values are abnormal in the first few days of life in the disease despite the absence of pregnanetriol from the urine The 17 OHP was also elevated but for the most part this was found only in the serum of the older subjects above 3 years of age with values generally in excess of 100 μg% The sensitivity of the method for 17 HP is 0 1 μg% and for PT and 17 OHP 0 5 μg% This method is rapid and convenient

D B Grant & S M Atherden (Harrow) *Circadian variation in plasma 17 hydroxyprogesterone in patients with congenital adrenal hyperplasia*

We have studied 4 patients with congenital adrenal hyperplasia (CAH) due to 21 hydroxylase deficiency using a simple protein binding technique to estimate 17 hydroxyprogesterone (17OHP) in serial blood samples Very high 17OHP levels were obtained in all 4 subjects One patient with simple virilizing adrenal hyperplasia studied before long term treatment showed a low evening 17OHP level and a high morning value Prompt suppression of 17OHP occurred after 50 mg i v hydrocortisone Two further patients with virilizing CAH also showed marked circadian variation in plasma 17OHP both on treatment and after therapy had been temporarily withdrawn A fourth patient with the salt losing form of the disorder showed high morning levels and low evening levels of 17OHP while on treatment with 100 mg cortisone acetate per day Treatment with dexamethasone produced a prompt fall in plasma 17OHP level

These findings suggest that relatively little adrenal suppression may be required during the latter part of the day in patients with CAH They also indicate that estimation of random plasma 17OHP levels may be of limited value in assessing therapy in CAH

H Glenpach & J Glatzl (Innsbruck) *Steroid pattern excreted in the congenital adrenal hyperplasia*

K E Petersen I Tygstrup & E Thamdrup (Copenhagen) *Isolated hypoaldosteronism—anatomical or biochemical defect*

We have earlier presented a boy with severe salt loss due to isolated hypoaldosteronism (resembling the cases first described by Visser et al) The aldosterone secretion was very low and remained low on sodium restriction—in spite of adequate stimulation from the renin angiotensin system as measured by renin in plasma The cortisol secretion was in the low normal range and secretion of corticosterone high

According to the findings the child was treated with sodium chloride and DOCA but he did not receive glucocorticoids He survived until the age of 18 months At the post mortem blocks of fat were found mimicking tissue located at the upper renal poles At histological examination it was impossible to find organised (adult) adrenal cortex

One year before the birth of our patient the parents lost another boy 5 weeks old Searching back it was possible to find and demonstrate the same pathological changes in this boy

The small lobules of cells found were impossible to distinguish from fetal adrenal cortex The picture does not seem to be like that in Vissers patient but seems like the so-called cytomegalic type of congenital adrenal hypoplasia

The testes in our patient were advanced in maturation—as judged from the ductus diameter the lumination the number of spermatogonia and the finding of precursors of Leydig cells The 17 keto steroid excretion was normal during the patient's life but plasma testosterone high on two occasions

N Josso (Paris) *Inhibiting activity of the bovine foetal testis on the rat foetal mullerian duct in vitro Comparative activity of seminiferous tubules and interstitial tissue*

Though it has long been known that the mullerian inhibiting hormone secreted by the foetal testis is not an androgen no information is available concerning the nature of the testicular cells which produce this substance Having demonstrated earlier the interspecific character of the mullerian inhibiting substance we have studied the effect of various components of calf foetal testicular tissue on the foetal rat mullerian duct in vitro Testicular tissue was collected from 17 foetal calves at a local slaughterhouse Two foetuses were over 200 days post insemination and the others under 160 days according to crown–rump length Seminiferous tubules and interstitial tissue were separated according to a modification of the method of Christensen & Mason and maintained in organ culture Mullerian inhibiting activity was assessed *in vitro* using the mullerian duct of the 14½ day-old foetal rat as target-organ Neither component of testicular tissue from the two older calf foetuses exhibited any mullerian inhibiting activity In contrast in the 15 younger foetuses whole testicular tissue inhibited the mullerian duct in 37 cases out of 38 and isolated seminiferous tubules did so in 22 cases out of 25 Isolated interstitial tissue inhibited the mullerian duct of only 3 tracts out of 18 and then contamination with tubular fragments was evident It is concluded that the mullerian inhibiting activity of the foetal bovine testis derives mainly from the seminiferous tubules

A Prader M Zachmann G Murset A Ferrandez & M Bambach (Zurich) *Treatment of excessively tall stature in children (girls and boys) with sex hormones The effect of estrogens and testosterone on growth and skeletal maturation*

In some tall children whose predicted mature height is excessive there is an urgent psychosocial indication and sometimes an organic one (progressive kyphosis) for treatment de

signed to reduce adult stature This can be achieved by the administration of sex hormones in small doses before or in larger doses after the onset of puberty The patients usually present in early puberty There are two main problems associated with this treatment First the accurate prediction of mature height is difficult and secondly there is the theoretical danger of inducing transient or permanent sterility as a result of gonadotropin suppression These problems have been studied and comparison has been made between the results of treatment with estrogens and testosterone

19 girls in puberty with bone ages of 12 to 14 years and a mean predicted height of 182 7 cm and 24 boys in or just before puberty with bone ages of 11 to 15 years and a mean predicted mature height of 199 5 cm have been treated 6 girls and 10 boys with tall stature served as control subjects The girls received ethinyloestradiol 0 3 mg (or stilboestrol 3 mg) daily for periods of 0 6 to 2 years with norethisterone added for a few days at monthly intervals The boys received 500–1 000 mg of a long acting preparation of testosterone esters intramuscularly at monthly intervals over a similar period A mean reduction in adult height of 3 2 cm in girls and 4 0 cm in boys was achieved after allowing for the tendency to overestimate mature height in the control subjects Bone age advanced approximately 2 years per year of chronological age During the first 6 months of treatment height velocity was greatly increased if the children had not yet reached the peak height velocity of adolescence and decreased if they had passed it After the first 6 months of treatment growth velocity was markedly lower than in the first 6 months though skeletal maturation continued unchanged The effect of estrogens was similar to but smaller than that of testosterone

Gonadotropin suppression was shown by the frequent occurrence of transient amenorrhoea after treatment and by the arrest or reduction in testicular volume during treatment The latter was reversed and full catch up growth occurred when treatment was stopped and in 5 boys examined later normal spermatograms were found in all cases

J R Bierich & D Schonberg (Tubingen)
Hormonal treatment of familial tall stature

Hormonal therapy was considered advisable in girls with a predicted adult height >180 cm in boys >190 cm if patients and parents asked for treatment Eleven girls aged 10 5 to 15 years and 3 boys aged 11 5 to 15 years were treated The steroids given were in girls conjugated oestrogens in high doses (Presomen® 6 × 1 25 mg per day) administered continuously together with 5 mg megestrol acetate daily for 5 days every 4 weeks Boys were given 250 mg testosterone oenanthate once in a month The average length of therapy reported here was 12 5 months

The mean increment of expected height without therapy was 10 cm in the girl patients With therapy this increment dropped to a mean of 4 5 cm In many cases growth came to a stop long before the epiphyseal closure Three girls who took a substandard dosage of the drug demonstrated unsatisfactory results In the boys too the results were not satisfactory with a monthly dose of 250 mg testosterone

When the mean increments of skeletal maturation and stature under oestrogens and testosterone during the first 12 months were compared a similar acceleration in bone age was found in both groups Longitudinal growth likewise accelerated under testosterone but diminished markedly under the oestrogens Apparently the oestrogens inhibit growth to an extent which surpasses their action on skeletal maturation

In order to elucidate the underlying mechanism we determined the plasma growth hormone (GH) with and without oestrogen treatment Neither insulin provocation nor investigation of the circadian rhythm of GH demonstrated any difference in the results in treated and untreated girls However there are reports regarding the suppression of the

serum sulphation factor in acromegalics with oestrogens Presumably the results obtained in our series were affected by similar mechanisms over and above the action of the steroids on the epiphyseal closure

All the girls showed regular vaginal bleeding every 4 weeks indicating hardly any side effects of the therapy In 2 girls in whom treatment could meanwhile be discontinued regular menstruation ensued

G H Newns (London) *Endocrinopathies in thalassaemia major*

The present paper reports 2 patients with Thalassaemia Major who developed Diabetes Mellitus and Hypoparathyroidism They were both retarded in growth and showed no signs of puberty

R S who was aged 22 when she died since the age of 1 1/ years received transfusions at approximately 6 week intervals At the age of 16 cataracts were noticed and a year later she developed tingling and carpopedal spasms The serum calcium was 4 0–5 0 mg/100 ml and the serum phosphorus was 8 3 mg/100 ml A tenfold increase in urinary phosphorus excretion occurred following intravenous parathormone There was marked growth failure Her height at 20 years was 146 5 (50th centile for a girl of 12 years) The epiphyses fused at 17 years of age There were no secondary sexual characteristics The urinary gonadotropin excretion (Bioassay) was very low The cortisol secretion rate and the ACTH stimulation tests were within normal limits The serum PBI was normal Plasma growth hormone after Bovril stimulation gave a peak response of 23 μu per ml Several months before death diabetes mellitus was diagnosed and it was necessary to give insulin Death occurred from hepatic and cardiac failure At autopsy there were extensive deposits of haemosiderin in the pituitary adrenal glands pancreas liver and heart muscle but none in the ovarian stroma Parathyroid tissue could not be identified

A C age 17 years had been given frequent transfusions at 6–8 week intervals since the age of 19 months Growth is retarded No puberty changes have developed At the age of 16 years she was found to have a diabetic type of glucose tolerance curve 6 months later she became overtly diabetic and required insulin Recently her serum calcium has fallen and the plasma parathormone level was found to be low She has recently developed tetany

Two other thalassaemic children have also developed diabetes and several others have shown an abnormal glucose tolerance curve with high fasting insulin levels

It is postulated that the massive deposits of haemosiderin in the endocrine glands gradually lead to destruction of the secretory cells with consequent partial or complete loss of endocrine function

C G D Brook (London) *Septo-optic dysplasia*

Published in *Brit Med J* 1972

K E v Muhlendahl & F Manz (Heidelberg) *Clofibrate and carbamazepines in the treatment of diabetes insipidus*

Four children with pitressin sensitive diabetes insipidus (d i) and one with nephrogenic d i were treated with clofibrate (CF) and carbamazepine (CA) Both substances are apparently relatively innoccuous and are currently widely used for conditions other than d i They are *administered orally*

CF and CA were ineffective in nephrogenic d i In the children with pitressin sensitive d i daily urine volumes decreased by 65 (40–83) with a concomitant augmentation of urine osmolality when CF was given at doses between 1 and 2 25 g per day CA 600 mg/day reduced daily urine volumes by 60 (50–70) In 2 children an adequate therapeutic effect (daily urine volumes constantly below 2 l/day) was seen only after combination of CF and CA which led to a reduction of urine output of 62* and 88 The minimal main

tenance dose of CF appears to be 30–50 mg/kg/day of CA approximately 10 mg/kg/day

All 4 children became independent of pitressin administration Both substances were tolerated without noticeable side effects The longest period of observation is 8 months

CF in healthy adult persons reduced urine flow to a highly significant extent during the first 2 hours after water loading

This study confirms previous reports on the treatment of 6 adult persons with d i with CF alone and on successful management of d i in children by CA It also demonstrates the beneficial effect of a combination of both drugs The mode of action by which these substances induce antidiuresis remains to be established

J W Finkelstein R M Boyar H Roffwarg & L Hellman (New York) *Synchronization of luteinizing hormone secretion with sleep at the initiation of puberty*

Luteinizing hormone (LH) was measured in plasma every 20 minutes for 24 hours in 14 children and adolescents in different stages of sexual maturation and in 5 adult men Polygraphic monitoring of nocturnal sleep was carried out simultaneously to precisely identify sleep onset wakefulness and sleep stages Prepubertal children showed no significant difference between sleep and awake mean LH concentrations Four boys and 2 girls in early puberty showed LH secretory patterns during the day that were indistinguishable from prepubertal children (2 5–4 5 mIU/ml) However with onset of night sleep there was an immediate increase of LH secretory activity that resulted in mean LH concentrations which were 2–4 fold higher (7 1–10 1 mIU/ml) than during wakefulness ($p <$ 001) In late puberty secretory episodes of LH could also be seen during waking hours However during sleep further augmentation of LH secretion occurred which resulted in mean LH concentrations (13 3 mIU/ml) which were significantly higher than during wakefulness (9 6 mIU/ml) When complete sexual maturity is reached in young adulthood, this sleep augmentation phenomenon disappears and there is no significant difference between awake and sleep mean LH concentrations By experimentally delaying sleep onset, synchronization of this LH secretory program with actual sleep was clearly demonstrated The number of LH secretory episodes during either night or day sleep corresponded to the number of REM NREM sleep cycles In attempting to correlate the initiation of LH secretory episodes with specific sleep stages it was noted that LH secretion was almost uniformly initiated during non REM sleep The point of downward deflection of the LH secretory episodes was also noted to occur in close proximity to or during REM sleep These data show that (1) with the initiation of puberty LH secretory activity becomes synchronized with sleep (2) during puberty secretion of LH is greatly augmented during sleep (3) enhancement of LH secretion with sleep is lost after sexual maturation is complete These findings constitute a new biological index for the early detection of puberty

J C Job P E Garnier J L Chaussain P Canlorbe & G Milhaud (Paris) *Effects of synthetic luteotropin releasing hormone on the release of gonadotropins in normal children and in some pathological conditions*

0 1 mg of luteotropin releasing hormone (LH RH) synthesized on solid phase has been injected I V and gonadotropins (FSH and LH) radioimmunoassayed in serum at −15 0 5 10 15 20 30 60 and 90 minutes with a double antibody method using LER 907 as standard reference

Control mean values and standard error of the means were obtained from 19 normal prepubertal children 11 boys aged 6 months to 12 years and 8 girls aged 3 months to 10 years LH response was higher in boys and FSH response in girls LH rise (of basal level) in

creased at puberty while relative FSH rise decreased mostly in girls

Of 9 hypopituitary dwarfs 6 with multiple deficiencies had no significant gonadotropin rise 3 with apparently isolated GH deficiency were in the normal range Of 8 subjects with isolated hypogonadotrophinuric hypogonadism (6 with anosmia) 5 had a normal or low normal response (primary hypothalamic defects?) and 3 did not respond at all 10 patients with simple delayed adolescence had a gonadotropic re serve in the normal prepubertal range

7 children with central isosexual precocity (idiopathic or tumoral) had high responses to LH RH whenever their basal levels of gona dotropins were not elevated 2 girls with isolated premature thelarche had a high FSH reserve

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R Illig S Pluznik M Bambach M Zach mann & A Prader (Zurich) *Studies on the pi tuitary responsiveness to synthetic luteinizing hormone releasing hormone (LH–RH) in chil dren*

The effect of LH–RH was studied in 77 male subjects 15 suffered from delayed adolescence or lack of sexual maturation (I) 30 from growth hormone deficiency (II) and 14 from primary testicular failure (III) As controls served 6 adult men 3 boys in early puberty and 9 boys before puberty aged 5–10 with unilateral cryptorchidism LH–RH was ad ministered intravenously usually in a dose of 25 μg/m Blood was taken at 0 10 15 20 30 60 and 120 minutes Plasma LH and FSH were determined radioimmunologically The results are expressed as ng LH LER 960 (1 mg = 4 620 IU) and as mIU FSH MRC 68/39 There were no untoward reactions The LH peak occurred after 20 to 30 minutes the FSH increase was irregular in time and duration In all *control* groups LH increase was highly significant with the following *base and peak values (mean $\pm$ 1 SD)* adult 1 55 $\pm$ 0 83 and 6 23 $\pm$ 1 33 puberty 1 17 $\pm$ 0 09 and 5 01 $\pm$ 1 14 before puberty 0 51 $\pm$ 0 10 and 1 73 $\pm$ 0 38 Base and peak values of FSH adult 2 69 $\pm$ 1 12 and 5 47 $\pm$ 1 21 puberty 4 38 $\pm$ 2 01 and 6 63 $\pm$ 2 89 before puberty 1 46 $\pm$ 0 53 and 4 07 $\pm$ 1 49

(I) In 6 boys with *delayed puberty* but be ginning sexual development (age 17–21 bone age < 14 ¹/) LH was 0 74 $\pm$ 0 25 and 5 69 $\pm$ 1 88 FSH 2 43 $\pm$ 1 15 and 4 9 $\pm$ 1 71 In 3 boys with similar development treated with testo sterone in physiological dosage the LH and FSH response was blunted 2 boys with in fantile genitalia (age 13 ¹/ bone age < 12 ¹/) had low base values but a peak comparable with controls in puberty In 2 boys with evi dence of and 2 other boys with suspicion of *isolated gonadotropin deficiency* the LH and FSH response was insufficient

(II) From 22 patients with idiopathic *growth hormone deficiency*—isolated or combined—13 had no LH response and the remaining 9 had a modest LH response comparable with pre pubertal children In 8 patients with growth hormone deficiency due to craniopharyngeoma and other tumors no LH increase occurred

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The response to LH–RH appears to be use ful to distinguish between benign delayed adolescence primary testicular and primary pituitary failure not only in puberty but also before puberty This confirms that the hypo thalamo pituitary gonadal axis is already active before puberty

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hormone (TRH) was expected to provide a more sensitive evaluation of pituitary thyroid function. TRH (100 or 200 μg) was injected intravenously to 35 children with various disorders like hypopituitarism, hypothyroidism, growth retardation etc. Patients without endocrine disorders served as controls. Plasma TSH and thyroxin (in some cases cortisol, blood sugar and growth hormone) were measured at 15 minute intervals during the first hour of the test and 2, 6 and 24 hours afterwards.

The mean basal TSH value was 3.1±1.8 μU/ml. The mean maximum value of 20.6±10 μU/ml was reached in the majority 15 minutes after injection of TRH. The maximum increase over the initial value was 17.5±9.1 μU/ml.

Extremely high levels and a prolonged elevation was found in untreated hypothyroidism. The either absent, diminished or normal TSH response found in hypothalamo-pituitary disorders possibly allows a distinction between hypothalamic or pituitary origin of secondary hypothyroidism. The TSH response in patients with clinical disorders compatible with marginal hypothyroidism was presented and the value of a TRH test for the diagnosis of otherwise undetectable mild hypothyroidism discussed.

K. A. Zuppinger, M. P. König & E. E. Joss (Berne): *The oral TRH/thyroxine (T_4) test in children with short stature*

In 14 normal children (4 ½–13 yrs) and 10 children with constitutional growth retardation (4 ½–14 ½ yrs) a significant rise of circulating total T_4 (Tetrasorb, Abbott) was found at different times (6, 9, 23 and 24 hrs) after oral administration of synthetic TRH (80 mg/1.73 m² at 0 and 12 hrs).

The same test was performed in a group of patients with Growth Hormone deficiency of various etiology. Seventeen of these (5–19 yrs) were euthyroid (basal T > 6.6 μg/100 ml) and fourteen (8–19 yrs) had various degrees of hypothyroidism (basal T_4 < 6.3 μg/100 ml). The results were as follows:

Group	Total Thyroxine (μg/100 ml) Basal mean ± S.E.M.	Maximal increase mean ± S.E.M.
I Normal children (14)	9.5±0.4	3.7±0.3
II Constitutional growth retardation (10)	9.2±0.4	3.8±0.4
III GH deficiency (17) euthyroid	9.6±0.5	3.1±0.3
IV GH deficiency (14) hypothyroid	3.7±0.6	1.6±0.4

No statistical difference was found between groups I, II and III. In group III only 2 patients had a diminished increase (1.5 and 1.5 μg/100 ml). In group IV 6 patients had a normal increase (1.9–3.8 μg/100 ml) indicating hypothalamic TRH deficiency. Three further patients of group IV upon prolonged oral TRH substitution (7 days) had a sustained increase leading to normalization of T_4. In 1 patient with an organic lesion prolonged TRH administration only led to a very small increase (0.9 μg/100 ml).

These results suggest that it is possible to distinguish hypothalamic from pituitary hypothyroidism by applying the oral TRH/T_4 test. No side effects of TRH were noted.

Y. Ingenbleek, R. De Meyer & P. Malvaux (Dakar and Louvain): *Iodine balance studies in protein-calorie malnutrition*

Twelve Senegalese children, aged from 18 to 30 months, with a peak incidence at 2 years were chosen for this survey. They presented all the clinical signs of uncomplicated protein calorie malnutrition. Failure of weight and height, behavioral changes, hair discoloration, diarrhoea and oedema in various degrees of magnitude. Dietary therapy was started immediately and consisted of increasing quanti

P C Sizonenko, A M Schindler A Cuendet & L Paunier (Geneva) *Endogenous follicle-stimulating hormone (FSH) in cryptorchid boys Presence of a feedback mechanism before puberty Its interaction on the HCG stimulation test*

1 27 bilateral testicular biopsies have been performed on 22 boys with cryptorchid or ectopic testes Spermatogonia were counted In bilateral cryptorchid testes (BCT) counts were low on both sides except in one case In unilateral cryptorchid testes (UCT) as well as in the unilateral ectopic testes (UET) counts were low on the affected side normal or sub normal on the unaffected side

Plasma FSH and LH were measured by radioimmunoassay In BCT boys at stage P1 of puberty mean FSH levels (2.6 ± 0.8) was higher than in normal prepubertal boys ($p < 0.001$) In UCT and UET boys, mean FSH level was 1.8 ± 0.7 not significantly different from normal boys Mean LH levels in all the 3 groups of boys at stage P1 were identical with normal values In stage P2 UCT and UET boys had normal plasma concentrations of FSH and LH

2 25 cryptorchid boys at stage P1 were submitted to a single injection of HCG (1 500 I U /m² i m) Plasma testosterone (TLS) was determined by a protein binding competitive method before and 3 days after the injection

There was a marked increase of plasma TLS on day 3 but no significant difference was observed between the 3 groups (BCT UCT and UET) No correlation was found between plasma LH on day 0 and TLS on day 0 and 3

A positive correlation was observed between plasma TLS and initial FSH levels Day 0 $r = +0.486$ $t = 2.664$ $p < 0.01$ day 3 $r = 0.576$ $t = 3.262$ $p < 0.01$ and between the increase of TLS (Δ TLS) after HCG $r = +0.477$ $t = 2.607$ $p < 0.02$ It may be concluded that (1) a supposed control mechanism between FSH and germinal cells is present before puberty (2) the testosterone response of the testes to HCG is dependent on the levels of endogenous FSH

W Hamilton (Glasgow) *G L C quantitation of thyroid hormones*

The *N* pivalyl methyl esters of iodinated tyrosines and thyronines are heat stable and do not decompose in the presence of atmospheric moisture They are prepared by adding to the dried samples 1 ml 25 anhydrous hydrochloric acid in anhydrous methanol followed by heating at 70 C for 30 minutes Thereafter the mixture is blown to dryness with nitrogen and solubilized with 20 µl dry methanol Pivalic anhydride (0 2 ml) and triethylamine (10 µl) are then added followed by heating at 110 C for 30 minutes Evaporation to dryness under nitrogen results in a white powdery residue which is ready for quantitative dilution with methanol for G L C A 5 feet steel column (I D 4 mm) with 2% loading OV 17 on Gas Chrom Q (100–120 mesh) was used in a Pye 104 G L C System Nitrogen (75 ml per minute) was the carrier gas and the temperature programmed from 225°C to 325 C with a 5° rise per minute Detection was by hydrogen flame ionization Good separation and peak heights were obtained for 1 µg amounts of MIT DIT T_3 and T_4

J Girard U Buhler P Kindler J B Baumann M Stahl & P W Nars (Basle) *On the assessment of pituitary thyroid function in children Plasma TSH levels with and without stimulation by synthetic TRH*

Plasma thyrotropin levels were measured in children with various clinical diagnosis including euthyroid goitre clear hypothyroidism suspected mild hypothyroidism growth retardation hypopituitarism etc

Extremely high levels of TSH were found in patients with proven primary hypothyroidism It has not been possible to evaluate moderate changes in TSH concentration however as the number of TSH estimations in our series was not large enough to allow a clear definition of normal limits

The pattern of TSH after intravenous stimulation with synthetic thyrotropin releasing

Diagnosis	Range irresp of age	n	Overlap with normal for age
Hyposomatotropism	0 13–0 53	6	0
Constitutional short stature	0 34–0 87	6	5
Primordial short stature	0 29–0 55	9	1
Achondroplasia	1 02 and 1 41	2	1
Cerebral gigantism	0 05–0 64	4	1
Obesity	0 86 and 1.29	2	1
Malnutrition	0 02–0 19	3	0

anism should also operate in the SM generating tissues where the intermediary substance should have a negative feedback effect on SM synthesis

J L Van den Brande M V L Du Caju C M Hoogerbrugge A M van Male J K Schouwstra & T Zurcher (Rotterdam) *Dose-response relationship between growth hormone (GH) somatomedin (SM) and other parameters of GH-effect*

Quantitation of GH-deficiency in humans has not been possible to date since dose–response relationships have been insufficiently elaborated In an attempt to begin establishing such a frame of reference this study was undertaken

Human growth hormone was administered to 6 children in weekly increasing dosages of 0 2 4 and 8 mg/m /day Two children presented with severe isolated GH-deficiency 2 were assumed to have partial GH-deficiency and 2 were so-called small normals SM levels fasting blood glucose and free fatty acids and the urinary excretion of OH proline calcium and phosphate were measured

The best dose–response curves were obtained with SM and OH proline as parameters This was particularly true with the severely GH-deficient patients who increased their SM levels from an average of 0 14 to 1 07 (potency ratio to a normal adult pool) and their OH proline excretion from 9 0 to 39 5 mg/m /day By fitting in the curves of the other children it seemed possible to approximate their deficiency quantitatively as well as to speculate about the mechanism causing their short stature

The other parameters investigated proved less relevant Glucose tended to rise if the baseline level was low and settled around 5 mMol/l in all cases FFA gave no consistent results The urinary Ca/P ratio increased in the GH-deficient patients and was similar in the others

K W Kastrup & H Andersen (Copenhagen) *Various aspects of the relationship between plasma IRHGH and somatomedin in normal and growth retarded children*

Somatomedin in plasma was measured by the method of Hall Intravenous injection of 2 mg HGH resulted in an increased production of somatomedin followed by an increased production of insulin Intravenous injection of insulin 0 1 U/kg resulted in an increased production of HGH followed by an increased production of somatomedin In hypopituitary patients and in a patient with dwarfism (Laron type) this was not the case The findings suggest an insulin mediated mechanism for the liberation of somatomedin and also a synergistic effect of insulin and somatomedin on the peripheral tissues

M Zachmann J A Vollmin & A Prader (Zurich) *Nitrogen retention studies using the stable isotope ¹⁵N*

The nitrogen retaining properties of human growth hormone (HGH) and of testosterone (T) have been studied extensively for many years HGH in small doses induces a more marked N retention in GH-deficient than in normal subjects T given for 5–6 days causes N retention in all subjects except patients with the classical testicular feminization syndrome The practical value of tests based on these principles is limited because they require a minimum of 10 to 14 days in hospital for balance studies Recently compounds con

ties of semi skimmed milk and a commercial mixture of oligopeptides and amino acids

Intake and excretion of iodine were measured on admission for 4 consecutive days with a total of 48 patient days under careful nursing supervision All the children improved satisfactorily and after clinical recovery 7 of them were submitted to comparative weight studies by the same sampling system and for a total of 28 patient days Measurement of iodine in food in urine and in feces, was performed by the humid method with Dade reagents and by triplicate determinations

Results show that in malnourished children iodine excretion always exceeds iodine intake This negative balance leads to the recognition that PCM is characterized by a continuous impoverishment of the thyroid gland s iodine content The reduced availability of halide may contribute to the explanation of decreased synthesis of thyroxine in kwashiorkor

On the other hand after a month of dietary treatment, the 7 reinvestigated children presented a reversed strongly positive balance This fact suggests that nutritional rehabilitation is accompanied by a progressive recovery of intrathyroidal iodine

J Waelkens (Amsterdam) *Iodide goitre in children treated for asthmatic bronchitis*

Potassium iodide is still occasionally prescribed in our country as an expectorant in the treatment of asthmatic bronchitis

Within 8 days after this iodine containing medication was stopped we measured the thyroid function (thyroid iodide clearance and absolute iodine uptake) in 20 children with asthmatic bronchitis 7 of whom had iodide goitre and/or myxoedema A significant difference between these two groups was found suggesting an inverse relation between inflow and outflow clearance in the early phase of iodine uptake The limit above which organification is disturbed is thought to be exceeded continuously

During the reinduction (300 mgKJ/m²) 5 out of 6 patients who had been reevaluated showed in contrast to the control group of asthmatic children a decrease of the serum hormonal iodine The plasma TSH levels were increased in 2 of them (21 4 and 27 5 μU/ml) and remained unchanged in 5 others and in the control group The adaptation to large doses of iodine seems to be based on an autonomous mechanism that becomes irresponsive in patients with iodide goitre

Relation to the metabolism of 3 5 cyclic adenosine monophosphate was further investigated As the phosphodiesterase activity in thyroid tissue fragment (homogenate 1 000 g supernatant) of a patient with iodide goitre was not decreased but increased (204 mU/mg DNA or 5 0 mU/mg protein) compared with controls < 120 mU/mg DNA or 2 mU/mg protein it seems probable that this was a consequence of increased 3 5 cyclic adenosine monophosphate production

The origin of the iodide goitre depends on a disturbance in the autonomous regulatory mechanism of the iodine uptake by the thyroid an overproduction of 3 5 cyclic adenosine monophosphate in these cases is considered to be independent of the TSH concentrations

M V L Du Caju & J L Van den Brande (Rotterdam) *Plasma somatomedin levels in growth disturbances*

A newly developed assay technique using porcine rib cartilage was applied to measure somatomedin (SM) levels in children with various growth disturbances and nutritional conditions Potency ratios (related to a normal standard pool) in 41 children whose data have been calculated to date are as follows Normals (*n* = 9 with ages 6 months to 13 yrs) range from 0 43 to 1 14 increasing with age ($y = 0\ 05x + 0\ 43$ $r = 0\ 92$)

Our present working hypothesis implies that SM stimulates the synthesis of a substance which in turn increases the rate of the metabolic processes leading to growth The mech

undoubtedly observed *after* a normal meal At the age of 16 months plasma insulin was first investigated and hyperinsulinaemia following glucose injection was detected

At 2 ½ years of age the child was referred to us because of its failure to thrive despite regular tube feedings (she refused to eat) and recurrent convulsive attacks coinciding with hypoglycaemia once complicated by temporary pareses The common causes of childhood hypoglycaemia were excluded High glucose assimilation coefficients between 4 0 and 6 0 and low fasting levels but excessive increases of plasma insulin following different betacytotropic stimuli indicated the existence of organic reactive hyperinsulinism which was suspected to be caused by diffuse beta cell hyperplasia The history of hypoglycaemia since birth the absence of leucine sensitivity low fasting levels of insulin and the reactive pattern of hyperinsulinaemia as well as a normal outcome of a coeliaca angiography seemed to rule out an autonomous islet cell adenoma

Severe dystrophy and the poor general condition of the girl prevented partial pancreatectomy Diazoxide treatment was instituted (5 to 18 mg/kg/d) and continued for now 3 ½ years During this period the child grew and developed well started to walk and to eat more or less properly Convulsions have never again been observed Reactive hyperinsulinaemia diminished while K values of glucose assimilation remained within the normal range Only side effects of treatment have been hypertrichosis and polyuria Blood pressure serum electrolytes and blood cell counts remained normal

Hence in severe hyperinsulinism of infancy a long term treatment with diazoxide seems to represent an alternative to surgical intervention

J R Ducharme J Letarte G Leboeuf & R Collu (Montreal) *Latent diabetes and gonadal dysgenesis*

Anomalies of carbohydrate metabolism and coexistence of autoimmune phenomena were searched for in 10 consecutively hospitalized cases of Turner syndrome by means of oral and intravenous glucose tolerance tests i v tolbutamide and conventional thyroid function tests The mean ± one standard deviation (and range) for chronological height and bone ages were respectively 15 7 ± 3 7 (7–21) 9 3 ± 1 9 (5–11) 11 5 ± 3 4 (4–16) By repeated peripheral leucocyte cultures only 4 showed gonosomal mosaicism (2 XO/XX 1 XO/XXr 1 XO/XX/XXX) Four (4) were receiving ethinyloestradiol while being tested Fasting blood sugar was less than 95mg in all but one case (110 mg) Using any one of 5 different conventional criteria of carbohydrate intolerance after oral/i v glucose loading the percentage of anomalies varied between 30 and 60 Insulin release was abnormally low in more than 50 of the cases Hypersensitivity to endogenous insulin was suggested in 4 of the 9 patients tested with tolbutamide By comparing 5 older subjects with a normal bone age (4 of which receiving ethinyl-oestradiol) and 3 younger patients with grossly retarded bone maturation it would seem that glucose disposal rate (Kt = 1 37 ± 0 47 vs 1 84 ± 0 34) shows an inverse relationship to growth hormone release during oral glucose load Indeed the mean fasting

the mean of $\frac{30 + 60}{2}$

and the mean maximal level of GH during the 3 hour test in the 2 groups were respectively 8 3 vs 1 8 4 6 vs 1 6 and 16 8 vs 6 5 ng/ml Thyroid antibodies by thyroglobulin hemagglutination and thyrotoxic gland complement fixation were positive in 7 of 9 patients and 4 had definite evidence of thyroiditis A weakly positive reaction against adrenal extract by complement fixation was obtained in 5 of 7 patients

taining the stable non radioactive isotope ^{15}N have been shown to be suitable for the study of protein metabolism in children

We have preliminary results concerning the effect of HGH (2 mg/m per day) and/or T (15 mg/m per day) on the excretion of ingested ^{15}N (NH_4Cl 50° ^{15}N 0 2 g/kg) in 9 prepubertal children (4 GH deficient and 2 endocrinologically normal boys 1 normal girl 2 siblings with the classical testicular feminization syndrome) which may be summarized as follows The ^{1} N balance under basal conditions (based on urinary ^{1} N excretion) was virtually identical in all subjects studied It was always positive and the mean value was $+10\,3 \pm 1\,25$ mg/kg After 2 injections of HGH the balance increased to a mean of +19 2 mg/kg in 4 GH deficient subjects while it remained unchanged in one normal subject Two of the normal subjects showed no change after 2 injections of T but there was a marked increment of the balance to +17 and +19 mg/kg in 2 normal subjects after 6 injections of T by contrast in the 2 cases with testicular feminization the ^{15}N balance did not increase at all after 6 injections of T These preliminary results suggest that HGH induces a N retention in GH deficient subjects only and that T for 2 days has no significance but T for 6 days has a marked N retaining effect in normal children In the testicular feminization syndrome there is no response after T treatment for 6 days

If these results are confirmed by further studies they allow an easy or even ambulatory test for diagnostic purposes in GH deficiency and in endorgan resistance to HGH or T

Z Laron Z Josephsberg & M Doron (Petah Tikva) *L Dopa stimulation of growth hormone secretion*

Full paper in press *Clin Endocr*

M Gourmelen M Donnadieu R M Schimpff H Lestradet & F Girard (Paris) *Advantages of ornithine infusion as growth hormone secretion test*

The test is performed as a conventional arginine test except that ornithine monochloride (25 g/1 73 m of a 6 25 solution) is used instead of arginine Blood samples are taken at −60 −30 0 +30 +45 +60 and 90 minutes

In 15 normal children ornithine induced a constant and marked rise of growth hormone The mean peak value (28 ng/ml) is higher than that is obtained with arginine (19 ng/ml) or insulin (18 ng/ml) and the variations about the mean of the individuals points are reduced Even if a nonspecific peak is noted before the infusion a further increase occurs in such a manner that no false negative response is observed The response is strictly localised at the points +30 and +45 (end of the infusion and 15 minutes later) This fact allows us to concentrate the assays to these two points as a screening test

Among 15 patients no response was observed in 6 well documented cases of panhypopituitarism and in 5 cases of isolated growth hormone deficiency confirmed by others tests In 4 cases the response was intermediate between hypopituitarism and normal children suggesting a limited pituitary reserve This fact was confirmed by others tests

In conclusion ornithine test seems to be a useful and reliable test for evaluation of growth hormone secretion

R Miething & B Weber (Berlin) *Long term treatment with diazoxide of infantile reactive hyperinsulinism*

P M female a former small for dates neonate (1 880 g 45 cm) with severe psychomotor and intellectual retardation showed persistent hypoglycaemia and convulsive spells during infancy first recognized early after birth Cerebral haemorrhage could not be excluded A long term corticoid treatment did not prevent further convulsions of which at least one was

for F response An insufficient response observed initially was followed by complete recovery or by either transitory or definitive fall to deficient values in the later course

In infants with a cerebral hemorrhage affecting the hypothalamus the associated hypoglycemia might be explained as a transitory or definitive impairment of hypoglycemia sensitive centres for both EPI and F regulation

P C Sizonenko M B Vallotton M Terraz & L Paunier (Geneva) Childhood hypoglycemia *Plasma glucose and renin response to 2 deoxy-d glucose A new diagnostic test for epinephrine insufficiency*

2-deoxy-d glucose (2 DG) a sugar analogue provokes in the cell a state similar to glucopenia In response to the induced glucopenia a consistent and pronounced increase in urinary epinephrine output is observed in subjects with intact adrenal glands or intact autonomic nervous system This increase of epinephrine secretion is correlated with a rise in blood glucose and plasma renin

In 5 control children aged 6 years 5 months to 8 years 7 months administration of 2 DG was followed by clinical manifestations of increased secretion of epinephrine A secondary hyperglycemia and a rise of plasma renin activity were observed

In 5 children of similar age presenting with idiopathic hypoglycemic episodes no clinical symptom of epinephrine secretion and no increase of blood glucose and plasma renin activity were detected

These results suggest that in patients with sporadic hypoglycemia the cellular glucopenia induced by 2 DG does not produce an epinephrine response This could be due to an absence of sensitivity of the autonomic nervous system to 2 DG or to a blockade in the efferent nervous pathways or in the adrenal medulla

This test would be helpful in the diagnosis of sporadic hypoglycemia due to the lack of epinephrine response to hypoglycemia

J L Chaussain P Georges & J C Job (Paris) *Tolerance to 24 hour fast in normal children and in ketotic hypoglycemia*

As a first step tolerance to 24 hour fast was studied in 56 normal children (25 boys 31 girls) aged 18 months to 9 years and in 10 children in the same age range with documented ketotic hypoglycemia In the control group mean blood sugar drop was significant after 8 hours and reached 52 ± 14 mg after 24 hours Individual values at 24 hours were normally distributed Ketonuria appeared in 40 control children and correlated to blood sugar In the ketotic hypoglycemic group mean blood sugar drop was more important the mean value at 24 hours being 27 mg% However this value as well as most individual patients values remained in the normal range ($m \pm 2$ S D) Ketonuria appeared in all hypoglycemic patients

In a second step more extensive data were obtained in 6 control children and 6 children with ketotic hypoglycemia submitted to the test

The response to glucagon was studied at the beginning and at the end of the fast The blood sugar and insulin mean responses to glucagon were similar in the 2 groups before fasting After the 24 hour fast these responses were still present but diminished in the control group and were blunted in children with ketotic hypoglycemia

Cortisol growth hormone and metabolic substrates were studied at hours 0 and 24 of the test No differences were observed in variations of serum growth hormone between the two groups A significant rise of mean plasma cortisol level (11 5 to 29 μg/100 ml) was present only in children with ketotic hypoglycemia Variations of NEFA lactate and pyruvate were similar in the 2 groups

Serum alanine dropped similarly in controls and patients By contrast all children with ketotic hypoglycemia except one exhibited at the end of the test a significant rise of branched aminoacids levels (+35% for valine

H K Akerblom V Koivisto & M K Kiviluoto (Helsinki) *Exercise induced metabolic events in uncontrolled diabetes*

The metabolic effects of exercise in uncontrolled diabetes are not sufficiently well known We approached the problem by using an experimental model of diabetes and the purpose was to look at the exercise related metabolic events in different degrees of insulin deficiency

Male Sprague Dawley rats were studied 42–96 hours after i v injection of streptozotocin (65 mg/kg) and before the start of insulin therapy Thirty one rats prior to exercise were classified as being either on the verge of or in diabetic ketoacidosis and the condition of 15 other rats, although clearly diabetic was less severe The untrained non fasted rats ran for 30 min in a rotating cage Blood specimens were taken from the retro orbital plexus of lightly anesthetized rats before exercise immediately and 2 hours after the end of it Some rats were killed either before or after the exercise and the pancreases were immediately removed for subsequent insulin extraction

Exercise caused a greater rise in blood lactate in diabetic than in control rats The rise was correlated to the decrease in blood pH in both groups Some of those rats which were acidotic prior to exercise died soon after completion of it and showed intensification of acidosis The observation is in agreement with the old clinical experience that exercise can be harmful in diabetic acidosis

Exercise lowered significantly the blood glucose and acetone bodies even in the severely diabetic rats Plasma glycerol rose significantly in both diabetic groups during exercise The rise in plasma IRI during exercise from pre exercise hypoinsulinemic levels was highly significant in the severely diabetic rats whereas the rise was of borderline significance in the less uncontrolled diabetic animals The phenomenon helps to explain the observed hypoglycemic effect of exercise Pancreatic IRI which prior to exercise was below the control level did not further decrease due to exercise

The observation of increased lipolysis despite a rise in circulating IRI warrants in vitro experiments to measure the processes of lipolysis and re esterification in adipose tissue in response to exercise in diabetic rats

R P Zurbrugg D Sidiropoulos & Hans Kaser (Berne) *Epinephrine and cortisol dysregulation in neonatal cerebral hemorrhage with hypoglycemia (NCHH)*

Infants with a central nervous system hemorrhage have an increased incidence of hypoglycemia Recent investigations indicate that a hormonal dysregulation of hypothalamic origin might be responsible for the impaired glucose homeostasis in some of these patients

Both adequate endogenous ACTH reserve and adrenal cortical function were demonstrated by vasopressin and synacthen tests respectively in all patients investigated Hypothalamic regulation of both cortisol (F) and epinephrine (EPI) were then evaluated by insulin tolerance tests (ITT) Some children with hypoglycemic attacks do not respond with an increase in EPI during the ITT indicating an adrenal medullary hyporesponsiveness as reported by various authors In such a group of patients we could recently in addition demonstrate an impaired F regulation The impairment of both EPI and F regulation in these patients might be independent of each other and of hypothalamic origin

With these observations as a background the possible importance of EPI and F as two of the glucose regulating principles were repeatedly evaluated in 3 infants with NCHH ITT was performed shortly after birth as well as 1/ 1 and 1 1/ years later Both the rise in urinary EPI excretion and of plasma F concentration were measured When first tested EPI response was virtually absent or insufficient in all 3 patients When reevaluated the EPI response became either normal or remained insufficient Similar results were found

This report illustrates the case of a 6-year-old boy born with severe myxedema The PBI was high (> 20 µg) nevertheless there was a [131]I uptake in the thyroid gland of 24 at 3 h The mother who was euthyroid had also a high PBI (> 20 µg) She had a normal [131]I uptake curve 15 months previously a hysterosalpingography with lipiodol was performed because of sterility On X ray examination there were no radio-opaque substances in the pelvis

We studied the boy at the age of 5 years and simultaneously some investigations were carried out with the mother The child was definitely hypothyroid though the PBI averaged 370 µg The BEI was 250 µg A PBI Jones and T 4 (Murphy Pattee) were in the hypothyroid range as was the T 3 uptake test TSH was elevated indicating primary hypothyroidism The mother had a PBI of 350 µg / and BEI of 240 µg PBI Jones T 4 and T 3 uptake test were normal The urinary excretion of I was low 49 µg I/24 h

In the serum of the mother and the child we *found* on TLC that the iodine-containing substance had not the R_f of iodothyronines and iodotyrosines Electrophoresis revealed that it migrated with the albumin band It only dialyses after extraction on a ion exchange resin

TLC on Sephadex indicates a molecular weight of about 12 000 *It is* not extracted with methanol chloroform Gaschromatographic analysis demonstrates no fatty acids in it After extreme purification *followed* by hydrolysis an aminoacid column chromatography revealed the presence of 15 aminoacids in the same proportion in both sera so mother and child have in their serum an iodinated small polypeptide After administration of a tracer dose [131]I to the mother radioactivity appeared in the polypeptide slower than in the hormonal fraction but the radioactivity in the polypeptide was still elevated until the 28th day During the following 2 years the concentration of this polypeptide decreased in the serum of both mother and child to a quarter of its initial value This suggests that there is a relation between the administration of lipiodol and the appearance of a small iodinated polypeptide in the serum of the mother which is slowly metabolized By transplacental passage it entered the foetal circulation

Whether it is directly responsible for the irreversible hypothyroidism in the child remains unknown

L. Clerc M P Koenig G Sauter U Walli & E E Joss (Berne) *Mental attainment of congenitally hypothyroid patients in relation to etiology quality of treatment and socioeconomic background*

The purpose of this study is to evaluate the role of various factors influencing the mental development of congenitally hypothyroid patients The cause of hypothyroidism time of onset and severity of the disease the quality of treatment the degree of intellectual stimulation from the surrounding (mainly family) and the interaction of these variables were analysed

All 35 congenitally hypothyroid individuals included in this study (age 4–24 yrs) had been properly diagnosed earlier and were under thyroid replacement therapy The IQ evaluation was carried out with the Kramer Test (Swiss variation of Binet Scale) in children below age 16 yrs and with the Hamburg Wechsel Test in those above age 16 yrs 28 pts had repeated IQ determinations The average interval between the first (IQ_A) and the last determination (IQ_B) was 4 8 yrs

There is no statistical difference between IQ_A and IQ_B However the difference between the IQ_B of well and poorly treated pts is statistically significant Children with onset of the disease before age 6 months and severe hypothyroidism have lower IQ (average 66) than pts with later onset and/or milder thyroid deficiency (average IQ 90) Within the group of pts with glandular ectopy and the group with metabolic errors there is a wide range of IQ values in accordance with the

+69% for leucine +79% for isoleucine) not observed in normal children

The data indicate the heterogeneity of ketotic hypoglycemia and the possibility of abnormal branched aminoacids metabolism occurring during fast in certain cases

M Karp Z Laron & M Doron (Petah Tikva)
Insulin secretion in children with constitutional familial short stature

A low insulin response to arginine stimulation test (peak value <40 μU/ml) was found in 80 children and adolescents with short stature For further evaluation oral glucose tolerance test (OGTT) was performed in 38 subjects (34 males and 4 females) and i v glucose tolerance test (IVGTT) in 10 subjects The clinical features of the subject studied were as follows (1) The ages ranged from 2 5 to 16 5 years (2) 23 of the families were of oriental Jewish origin (3) The height of all the parents was below the normal mean (4) The height of the subjects was below the 3rd percentile and the average skeletal age was 3 years retarded (5) The subcutaneous skin fold thickness was below the normal standard

All the subjects showed normal growth hormone response to insulin and arginine stimulation tests Low insulin response to OGTT as compared with normal controls (peak <60 μU/ml) was found in 30 subjects and in 20 of them it was in the range found in children with juvenile diabetes No glucose intolerance was observed The K value in IVGTT was normal in 9 subjects and low insulin response (<50 μU/ml) was found in 5 It is hypothesized that insulin acting synergistically with growth hormone has an important role in normal growth The cause of the low insulin response in the subjects studied may be attributed to the lean body mass which is possibly related to nutritional and environmental factors

J J van der Werff ten Bosch (Rotterdam)
Desirability of considering planning and execution of long term studies into the fate of paediatric endocrine patients

Over the past 20 years clinical endocrinology has undergone profound changes with enormous advances in diagnostic and therapeutic methods and knowledge One result is that many individuals are now going through life after endocrine interventions early in life and (or) undergoing prolonged or continuous endocrine treatment from early childhood on Many such individuals get a regular follow up but that is often limited to the making of observations directly related to the original disorder only and is often terminated after having attained the original goal of the treatment It seems to me that both for practical purposes and for academic reasons it might be rewarding to carry out long term assessments of particular groups of patients Our Society could be useful for the formulation of minimal programmes and for the undertaking of joint studies on problems that cannot be resolved on the basis of the small size of the group of patients available to any one local group Examples of long term studies requiring joint action from my own sphere of interests are

1 prenatally virilized girls age at menarche fertility age at menopause psychological and social development comparisons between subgroups according to sex assignment age and type of treatment degree of virilization at birth

2 precocious puberty natural history of complete and incomplete forms gonad histology blood levels of hormones onset of maturity in terms of these and other parameters fertility

3 height prediction short and tall children at different ages validation of available and (or) preparation of new standards

A Elewaut C Van Nevel & C Hooft (Gent)
Presence of a iodopeptide in the serum of mother and hypothyroid child several years after hysterosalpingography with lipiodol

Age 16 years	Urinary			Plasma			FSH/LH
	17 KS	17-OH	Aldost	T	DHEA	Δ4	
Baseline	5.5	1.0	0.55	0.19	0.10	0.17	13.2/8.8
Post ACTH	4.1	1.0	0.27	0.14	0.09	0.13	—
Decadron	3.8	0.54	1.1	0.17	0.09	0.15	—
Norlutin	3.6	—	0.93	0.05	0.05	0.12	12.8/8.0
HCG	5.2	—	0.50	0.30	0.17	0.35	—
Post-ovarian surgery*	4.9	—	—	0.08	0.13	0.25	—
Normal values				0.004–0.07	0.14–1.25	0.05–0.33	

Endocrine Studies Post Adrenalectomy and Post Adrenalectomy plus Wedge Resection of Ovaries and Removal of Hilus Cell Tumor

narche occurred at age 12 years Over the following years the patient became progressively more pigmented suggesting Nelson's syndrome In addition amenorrhea loss of breast tissue and virilism occurred Pelvic exploration at age 16 revealed bilateral polycystic ovaries and a left hilus cell tumor (1 × 1 cm) Plasma testosterone (T) prior to adrenalectomy had been elevated (0.11 μg) and did not increase after ACTH administration Following adrenalectomy and in the presence of virilism plasma T was in the low adult male range (0.17–0.24 μg) and did not stimulate with ACTH However Norlutin caused a fall to ½ baseline values while HCG doubled the value Laboratory data are shown in the table above

At surgery T was extremely high in the ovarian vein plasma 9.3 μg on the side of the tumor and 4.9 μg on the other side Ovarian vein levels of DHEA (2.5 μg and Δ4 (8.3 μg) were equal bilaterally After removal of the hilus cell tumor and bilateral wedge resection of the polycystic ovaries plasma T decreased to 0.08 μg DHEA and Δ4 did not change significantly In summary this case allowed the study of ovarian virilism in childhood in the absence of an adrenal contribution to androgen production The primary etiology of the adrenal and ovarian disorder may reside in a disturbance of pituitary secretion of ACTH and gonadotropins

R. Stanescu V. Stanescu & P. Maroteaux (Paris) *Histochemical and microchemical studies on human growth cartilage in foetuses and newborns*

Microchemical determination of hexosamines and hydroxyproline were performed in isolated histological zones from sections of tibial epiphyseal plate obtained from human foetuses newborns and from older children Alternate sections were used for histochemical stainings

The case material included (*a*) 7 human foetuses selected according to the usual criteria (*b*) 14 newborns who died soon after birth and were divided in two subgroups children dying within hours after birth due to obstetrical accident or unknown causes and without important or specific pathologic findings newborns with more important or more specific pathologic findings (*c*) 4 children aged 5–11 years who died after traffic accidents

There is a decrease of cellularity and of hexosamine concentration and an increase of matrix and of hydroxyproline concentration with age The septa between the columns become thicker and nonmetachromatic The metachromasia of the *resting zone* is less strong in the older children A system of canals with vascular anastomoses across the plate is well developed in the late foetal and in the newborn period

In all the age groups there is an increase of hexosamine and a decrease of hydroxyproline

variable amount of hormone produced by the defective thyroid gland during the crucial period of CNS development In individuals from underprivileged socioeconomic groups (low income disturbed family life with neglected children) insufficient treatment was more frequent and the average IQ lower than in pts with better socioeconomic conditions

A K Slob C E Snow & E de Natris Mathot (Rotterdam) *Lack of behavioral deficits following neonatal food deprivation (without social or maternal deprivation) in the rat*

It is widely believed that in the human malnutrition early in life affects mental development and intelligence in adulthood Actually there is no good evidence available since it is impossible to isolate malnutrition from maternal social and educational deprivation in human populations So far animal studies have also failed to yield solid evidence for this belief All studies of the effects of early undernutrition on behavior in adult rats for instance have confounded underfeeding with maternal deprivation or membership in a large litter In the present experiment an attempt was made to control social context, i e littersize and maternal care Food deprivation from day 1 till day 25 was achieved by placing the rat pups for 12 hours per day with a foster mother who would display all maternal behaviors except lactating Littermate controls remained with the lactating mother at all times Litter size was kept at a standard size of 8

In adulthood beginning on day 75 subgroups of the animals were tested for (*a*) exploratory behavior and emotionality in the open field (*b*) motor ability on an elevated runway (*c*) baseline activity in a residential plus maze (*d*) food motivated learning capacity in the Hebb Williams maze and (*e*) shock motivated visual discrimination learning using a Thompson box

The food deprivation regimen produced animals which were significantly lighter and smaller in adulthood at 120 days of age ($p < 0\ 001$) Eye opening auditory reflex, and vaginal opening were significantly retarded in food-deprived animals ($p < 0\ 02$) In contrast to previous findings the experimental animals in the present study did not differ from controls in emotionality and exploratory behavior in motor coordination and in learning ability However food deprived animals were more active than controls in the residential plus maze Females showed less effect of food deprivation on body growth but a much greater effect on activity than males

These findings suggest that early undernutrition when not confounded with social and maternal deprivation may have more restricted effects on adult behavior than has been previously believed

S Korth Schutz E J Siegal I R Merkatz & M I New (New York) *Endocrine evaluation of ovarian virilism in a child adrenalectomized for Cushing s syndrome*

Endocrine studies were carried out over a 6 year period in a girl who presented with Cushing s syndrome and acanthosis nigricans required bilateral adrenalectomy and subsequently became markedly virilized and hyperpigmented At age 10 years when she was initially evaluated for Cushingoid features the laboratory values did not confirm the diagnosis A normal diurnal variation of plasma cortisol was present One year later when Cushingoid features and acanthosis nigricans were markedly aggravated the diagnosis of Cushing s syndrome was confirmed by the lack of diurnal variation of the elevated plasma cortisol and ACTH levels Suppression of plasma cortisol and 17 hydroxycorticoids was prompt and complete on 8 mg of dexamethasone whereas urinary 17 KS were only partially suppressed Two hyperplastic adrenals weighing 8 5 and 11 5 g were removed Subsequently the acanthosis and Cushingoid features vanished and within six months after adrenalectomy me

Group	Before Ca		After 1 min		After 3 min	
	Glucose mg	IRI μU/ml	Glucose mg	IRI μU/ml	Glucose mg	IRI μU/ml
A	95 ± 4	46 ± 9	78 ± 4	101 ± 10	77 ± 4	49 ± 10
B	208 ± 18	76 ± 9	186 ± 0	241 ± 48	180 ± 18	148 ± 39
A + B	159 ± 18	62 ± 7	141 ± 48	183 ± 32	140 ± 17	106 ± 25

Mean ± S E M

calcium glucose and the immunoreactive insulin were determined According to various glucose concentrations the results of the Ca infusions were divided into three groups In *group A glucose was less than 110 mg* ($n = 8$) in group B glucose was above 110 mg ($n = 11$) Group A + B represents the mean of all tests ($n = 19$)

Calcium rose significantly in each group while glucose decreased at the same time but this decrease was significant in group A only Insulin increased in all three groups significantly The greatest increase was in group B with the highest starting glucose levels Glucose infusions alone showed different results Insulin release was stimulated in 3 out of 5 infants while 2 infants showed no response

The results suggest that the mechanisms by which glucose and calcium stimulate insulin secretion are closely correlated but not identical in the human newborn

M Van der Schueren Lodeweyckx R Wolter E Eggermont & R Eeckels *Plasma growth hormone in coeliac disease*

Insulin tolerance tests have been performed in 60 normal prepubertal children and in 9 patients with active coeliac disease of the same age group All coeliac patients fulfil the diagnostic criteria set up by the European Society of Paediatric Gastroenterology Blood samples are taken before and 15 30 45 60 90 and 120 minutes after the injection of crystalline insulin (0 1 U/kg) Growth hormone is measured by the double antibody radioimmunoassay with purified Wilhelmi human growth hormone (HS705A) as standard Blood glucose is determined by the glucose-oxidase method and plasmacortisol by competitive protein binding

In the normal children the maximal value of plasma growth hormone is 17 4 ± 4 9 ng/ml ($M \pm 1$ S D) In coeliac patients the maximal *response is* 5 9 ± 2 6 ng/ml ($M \pm 1$ S D) and the maximal value is below the mean minus 2 S D in 7 out of the 9 patients In the patients with *glutenenteropathy* the fall of blood glucose is comparable to that of normal children but there is no responsiveness to hypoglycaemia In coeliac disease plasmacortisol rises normally during the first 60 minutes but remains high for another hour

These results suggest that glutenenteropathy results in some degree of pituitary hypofunction It shows also the necessity of excluding coeliac disease whenever the release of growth hormone is impaired

C Dacou Voutetakis M Constantinidis & N Matsaniotis (Athens) *Diurnal variation of growth hormone in obese children*

The spontaneous diurnal peaks of plasma G H were examined in 6 obese children of normal height and without central nervous system disorder and 6 nonobese endocrinologically normal children Blood specimens were obtained hourly during the 24-hour period

G H was determined by a double antibody radioimmunoassay The mean hourly level of G H in obese children was 2 20 ± 0 9 ng/ml In the control group the corresponding value

concentrations from the resting to the columns zone

In the newborns the change is less marked and the values obtained had a larger dispersion due probably to the lack of homogeneity of the group In several newborns with important pathologic findings on the contrary a decrease of the hexosamine concentration was found in the columns zone as compared with the resting one

The data obtained suggest that the permeability of the growth cartilage is higher in the foetal and in the newborn period than in older children The distribution of the oxygen tensions may be different due to a different vascularization pattern

The possible significance of these results for the age dependent variation in the responsiveness of the growth cartilage to somatomedin is discussed

F Bayard (Toulouse) *25 hydroxycholecalciferol in human plasma*

A radio ligand assay for the measurement of 25 hydroxycholecalciferol (25 OH D_3) in human plasma has been devised using the plasma of an osteomalacic man as a source of binding proteins Plasma concentration in normal subjects was 1 50 ± 0 40 (mean ± S D μg/100 ml) The determination of the rate of catabolism of tritiated 25 OH D_3 allowed the evaluation of the exchangeable pool at 200 μg

The 25 OH D_3 plasma concentration was low in babies with nutritional rickets (0 62 ± 0 16 $p \sim 0\ 005$) in adults with malabsorption syndrome and osteomalacia (0 33 ± 0 22 $p < 0\ 005$) and in patients on long term corticosteroid therapy (0 51 ± 0 26 $p < 0\ 005$)

In patients with chronic renal failure before or on hemodialysis, there was a good correlation between the 25 OH D_3 levels and the form of bone disease presented by these patients There was also a good correlation between their 25 OH D_3 plasma concentration and their calcemia suggesting that 25 OH D_3 is an important factor in bone mineralisation and metabolism

B T Rudd P J M Watney & W R Butt (Birmingham) *The assay of human plasma parathyroid hormone (h PTH)*

A study has been made of factors which influence the reliability of h PTH measurements by radioimmunoassay

(1) Two products Medical Research Council bovine PTH (b PTH) and highly purified b PTH from Wilson Laboratories Chicago were iodinated with ^{1} I Considerable iodination damage occurred which was independent of the purity of the b PTH

(2) Two stage purification of the iodinated label by adsorption onto microfine silica followed by sephadex filtration (G100) is necessary to remove damaged material

(3) There is marked inhibition of binding of the label to the first anti b-PTH/h PTH antibody in the presence of human plasma proteins The inhibition is proportional to the quantity of plasma protein in the incubation medium Even when highly purified label is prepared it is subject to incubation damage in the double antibody technique and in the method which separates free from bound label using dextran coated charcoal in place of a second antibody

(4) Provisional data on h PTH levels during the course of pregnancy are presented which show that only relatively high levels can be measured by the techniques currently available and only then if undamaged purified b PTH label is prepared

E Heinze R Fussganger & W Teller (Ulm) *The effect of calcium and glucose on insulin secretion in newborns*

Calcium uptake by β-cells is associated with the release of insulin Therefore the insulin response to calcium injections (2 ml 10 % calcium gluconate) was tested in newborns and compared with the effects of glucose injections (0 33 g/kg) On the occasion of an exchange transfusion with citrated blood via the umbilical vein eight newborns were studied Before 1 and 3 minutes after the injections

We report on a newborn male infant who presented with a goitre and typical signs of severe hypothyroidism at the age of 3 weeks Because of Rh incompatibility this child received two intra uterine transfusions in the 29th and 32nd week of gestation During these procedures X ray contrast medium was instilled into the amniotic sac as well as into the peritoneal cavity of the fetus and into the gluteal musculature of the fetus The child was therefore challenged with a total amount of approximately 12 g of I At the time of diagnosis PBI and BEI determinations were unmeasurably high due to heavy contaminations with iodine containing material in the plasma Under treatment with thyroxine the goitre as well as the hypothyroidism disappeared Because PBI was still very high at the age of 15 months thyroxine therapy was continued up to the age of 18 months The child remained euthyroid and the goitre did not recur To prove the diagnosis of iodide induced hypothyroidism and goitre the patient was exposed to 23 mg I/day for 10 weeks at the age of 2 3 years Within 10 weeks a moderately large goitre and minimal signs of hypothyroidism had reappeared The child returned to normal after discontinuing iodine medication

J M Crocker & B T Rudd (Birmingham) *A study of the distribution of enzymes concerned with the biosynthesis of oestrogens in human term placentae*

Studies are being carried out on oestrogen biosynthesis by teased villi prepared from fresh term placentae Using (7α-^{3}H) D hydroepiandrosterone sulphate in the medium as substrate labelled DHA Androstenedione Oestrone and Oestradiol have been isolated quantitatively by thin layer chromatography Preliminary studies indicate that there is an uneven distribution of the enzyme activities concerned with successive biosynthetic steps in the formation of oestrogens throughout the body of the placenta

N M Drayer & J G Ackers (Petten) *Total body potassium during growth hormone administration*

A T A Farzekas J Homoki & W Teller (Ulm) *Tissue cortisol before and after sexual maturation in guinea pigs*

Following sexual maturation there were more steroid fractions in higher quantities except corticosterone in tissues of female rats compared with males These findings prompted us to determine cortisol concentrations of nine different peripheral tissues including adrenals in colored guinea pigs of the same breed and both sexes before ($n=12$) and after ($n=12$) sexual maturation In 0 3–0 6 g of different tissues homogenized in ethanol cortisol was assayed by a modification of competitive protein binding method

Before sexual maturation cortisol levels in female guinea pigs were found between 1 31–82 8 μg/100 g wet weight of tissues (liver 7 21 μg kidney 11 68 μg muscle 2 14 μg spleen 2 36 μg lung 3 79 μg heart 3 67 μg brain 1 31–1 51 μg blood 15 26 μg adrenals 82 8 μg) After puberty the tissue levels of cortisol (except in the liver) were 18 3–52 0 lower compared with prepubertal levels Liver tissue revealed 36 4 (male)—72 1 (female) higher levels of cortisol after puberty There was no significant difference between female and male animals either before nor after puberty

The differences in cortisol concentrations of peripheral tissues at various stages of sexual maturation could be explained by different cortisol metabolism or variability of general tissue metabolism before and after puberty

D Gupta A Attanasio & E McCafferty (Tubingen) *Simultaneous radio-immunoassay of the androgens and oestrogens in the peripheral plasma of children during pubertal changes*

A reliable and simultaneous radio immunoassay for the peripheral plasma androgens (testosterone & dihydrotestosterone) and oes-

was 5 22±3 1 ng/ml The difference between the two groups was statistically significant with $p = 0\ 05$ During sleep high peaks of G H were observed in both groups as expected but were lower in the group of obese children Thus the higher values in the obese subjects did not exceed 8 ng/ml while in the controls values above 20 ng/ml were detected

The diurnal variation of plasma G H also occurs in obese children but the peaks are lower than normal The process of sleep activates the hypothalamic pituitary axis in the obese subjects as well but less efficiently than in the controls This test can be applied to exclude hypophyseal insufficiency whenever obesity is associated with short stature The cause of lower levels of plasma G H in obesity remains obscure

E P Trias, L L Levitsky M S Grossman & S Raiti (Baltimore) *Comparison of HGH oxandrolone and combined therapy in idiopathic hypopituitarism Hand Schuller Christian and Prader Willi syndromes*

Six (6) idiopathic hypopituitary patients (IHP) one with Hand Schuller Christian Syndrome (HSC) and one with Prader Willi Syndrome (PWS) were studied Pretreatment growth rates were measured Each received Human Growth Hormone (HGH) at 2 IU × 3 per week for 8 months The HSC patient was given 4 IU × 3 per week Oxandrolone (0 25 mg/kg/day) was given for 8 months Both HGH and oxandrolone were given for 8 months Between each study period neither hormone was given for 4 months Bone ages (B A) were measured at 12 month intervals

At the commencement of the study the height ages (H A) were delayed by 3–8 years and B A by 1 7–8 0 years in the 6 IHP patients The HSC patient showed H A and B A delays of 6 5 and 4 5 years The PWS patient showed H A and B A delays of 6 0 and 2 5 years All pretreatment growth rates were less than 4 6 cm/year (mean of 3 7 cm per year for IHP patients) except for the PWS patient who was growing at 5 8 cm/year Her I Q was 40

During HGH therapy 5 of the 6 IHP patients grew at 7 6–11 1 cm/year No growth acceleration occurred in one IHP and the PWS patient The HSC patient grew little When HGH was stopped the PWS and 4 of the 6 IHP patients grew less well than before (mean = 2 2 cm/year) With oxandrolone 4 of the 6 IHP and the PWS patients grew at 6 5–10 2 cm/year Two IHP patients did not respond After therapy the mean growth rate for the IHP patients was 3 2 cm/year (similar to pre study growth rate) During combined therapy all IHP and the PWS patients grew at 2–4 times the pre study rate, but not better than when either hormone was used alone The HSC patient responded poorly to all 3 forms of therapy

The B A accelerated at 1 1 years per year of study in all IHP patients except 2 (whose acceleration was 1 5 years per year) After 3 study years all bone ages were still significantly delayed by 2–6 years The B A acceleration for the HSC patient was 0 7 year per year and for the PWS patient was 2 2 years per year

Alternate therapy can be used in IHP Growth acceleration from HGH therapy approximated that from oxandrolone treatment though some responded better to one or to the other Combined therapy did not significantly potentiate the growth The B A remained significantly delayed The HSC patient responded poorly The PWS patient responded to oxandrolone but not to HGH and also showed marked B A advance Oxandrolone doses of 0 15 mg/kg/day are being used in further studies

PAPERS READ BY TITLE

U K Buhler J Girard & G Stalder (Basel) *Congenital iodide goitre and hypothyroidism due to intra uterine application of iodine-containing contrast medium*

Four of the patients have been troubled with moderate or severe keratitis Two have alopecia

Three of the hypoparathyroid patients have had severe episodes of intestinal malabsorption Two of these also have pernicious anaemia The third died of acute hepatitis at 10 years of age and another boy had this disease at 3 years of age

D C L Savage C C Forsyth J Cameron & E McCafferty (Dundee) *Excretion of individual 17-oxosteroids and corticosteroids in the urine of children of small stature*

The excretion of individual adrenal androgens and corticosteroids in the urine of children whose height and weight were below the 3rd percentile were compared with the results in normal children

Two groups each consisting of 20 small children have been studied their ages ranged from 6 months to 16 years The children in the first group had no apparent abnormality and their small stature was probably genetically determined The second group of children had a variety of chronic illnesses (mucoviscidosis malabsorptive disorders asthma renal disease) which were thought to have contributed to their small stature

The excretion of the adrenal metabolites in these two groups of small children are not different from each other but in both groups they are significantly lower than in the normal children When the results are expressed against bone age rather than chronological age the difference between the small and normal children though less marked is still apparent The lower values for the adrenal androgens particularly in the older small children are partly due to delayed puberty The lower levels of cortisol metabolites are less easily explained since when corrected for body weight the results remain lower than those of normal controls In those small children whose adrenals were stimulated with ACTH (20U i m BD for 3 days) or who were given metyrapone (500 mg × 6 doses) normal responses were obtained

These results show that there is no difference in the quantitative or qualitative excretion of adrenal cortical steroids in children whose small stature is either constitutionally determined or secondary to chronic illness but that both groups have a lower level of excretion than normal children The basal levels suggest a degree of adrenal hypofunction but this seems unlikely in view of the children s normal response to ACTH stimulation and metyrapone It is possible that these subnormal levels reflect a lesser physiological need but it may be that there is an altered metabolism of the adrenocortical steroids in some of these children

D Schonberg (Tubingen) *Plasma growth hormone in cerebral giantism Laurence Moon Bardet Biedl syndrome Bloom syndrome and in Fanconi anemia*

In some syndromes with growth disorders the role of growth hormone (GH) is still uncertain GH in plasma has been measured under various conditions with a modified double antibody technique

Three children presented the typical clinical signs of cerebral giantism The response of GH to insulin induced hypoglycemia was normal in all children but maximal stimulation after infusion of arginine was subnormal in 2 cases (4 1 resp 3 9 ng/ml) Peak levels of the circadian secretion of GH were normally distributed but rather low compared with two matching controls with constitutional overgrowth

Of 2 children with Laurence Moon Bardet Biedl syndrome 1 patient with normal height for age showed a normal response of GH to insulin induced hypoglycemia The other child with retarded growth (S D − 2 2) responded subnormally to arginine (3 3 ng/ml) The adrenal function was impaired as well

trogens (oestrone and oestradiol) has been developed to study the roles of these hormones in children during their adolescent growth. Plasma volumes of 2 ml *from each of 34 children at sexual maturation stages between 3 and 5* were used for analysis.

The alkaline plasma was extracted with diethyl ether and the extract after being washed with glacial acetic acid and water was applied to a 10 cm Sephadex LH 20 column. The chromatogram was developed with an eluting solvent of *n* hexanemethanolethyl acetate 95 5 5 (v/v) and the above mentioned steroid hormones were isolated into 4 individual fractions without any measurable cross contamination. Following the evaporation of the eluting solvent the dried material was incubated in suitable system with antibodies for testosterone and oestradiol for the androgens and oestrogens *respectively* at 4 C for 3 hr. Charcoal dextran suspension was used for the separation of the free from the bound steroids. Radioactive steroids were used for the correction of losses which were on average 32° (range 21–38°). The accuracy and the precision of the method was satisfactory. The sensitivity was 10 pg per sample.

K F Hanssen H Andersen & K W Kastrup (Copenhagen) *Immunoreactive growth hormone in urine of normal children and in patients with hypopituitarism and other forms of growth retardation*

86 normal children and children with various disorders of growth have been investigated by determining 24 hour urinary immunoreactive growth hormone (IRHGH) together with plasma immunoreactive growth hormone concentration during insulin induced hypoglycemia. Urinary IRHGH increased with chronological age in both normal and growth retarded children. Urinary IRHGH was well correlated to body surface. A positive correlation was shown between urinary IRHGH and the integrated plasma growth hormone response during insulin induced hypoglycemia ($r = 0.47$ $p < 0.01$). Hypopituitary children excreted significantly less IRHGH in urine per m than normal children ($p < 0.005$). With a few exceptions no difference in urinary IRHGH/m between normal children and children with non pituitary growth retardation was shown.

J Perheentupa & H Hiekkala (Helsinki) *Twenty cases of the syndrome of autoimmune endocrinopathy and candidiasis*

The series consists of 12 females and 8 males and includes a set of four siblings another of three and two pairs of brothers. All these subjects have mucocutaneous candidiasis which has been apparent in some from early infancy and several years prior to other manifestations. In others, it was documented at latest at the time of the first diagnosis of an endocrine disorder. Nineteen have an endocrinopathy. Hypoparathyroidism has been diagnosed in seventeen at the age of 1.6–12.0 years (mean 4.8 years). It is the only endocrine disorder so far in 7 patients but 3 of these have circulating antibodies against the adrenal cortex. Eleven patients have manifest adrenocortical deficiency diagnosed at the age of 5.0–12.0 years (mean 8.3 years). One of these has had definite decrease in the reserve capacity of cortisol secretion for one year with intact tolerance of salt restriction. Another had manifest salt loss for 0.6 year before a decrease in the cortisol reserve became demonstrable. Of the 9 patients with both hypoparathyroidism and Addison's disease hypoparathyroidism was diagnosed from 0.6 to 6.0 years earlier in seven coincidentally with the Addison's in one and a month after this in one patient. Two of the girls have primary ovarian failure with demonstrated lack of ovarian cortical tissue and three others have high titre of circulating antibodies to the cells of corpus luteum. The oldest patient alone has primary hypothyroidism manifest since age 19. She also has hypoparathyroidism, Addison's disease and ovarian failure. At least two others have significant titres of thyroid antibodies.

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One child with Bloom syndrome had a subnormal response to insulin with a late rise of GH to 14 ng/ml after 210 min The nitrogen retention test was subnormal GH therapy (Kabi) has not been successful so far

GH stimulation in a girl with Fanconi anemia and severe growth retardation (S D −5) was in the range of hypopituitary dwarfs GH therapy (Kabi) seems to have a favourable effect both on growth and on haemopoiesis

C G Bergstrand

BOOK REVIEWS

B Modan *The polycythemic disorders* Charles C Thomas, Springfield Illinois 1971 177 pp US $76 00

The main part of the book is concerned with polycythaemia vera an extremely rare disorder in children The author seems to accept only four cases of those reported in literature The obscure nature of the disease its clinical and laboratory manifestations and its treatment are clearly and fully described

Of greater interest to paediatricians are the introductory chapter on regulation of erythropoiesis and the two short chapters on secondary and benign polycythaemias As pointed out by the author all these diverse conditions should rather be termed erythrocytoses because in contrast to the findings in polycythaemia vera the abnormalities are confined to the red cells and their precursors

The book is of little value in an ordinary paediatric library

Stig Sjölin

W J W Sharrard *Paediatric orthopaedics and fractures* Blackwell Scientific Publications Oxford 1971 1 126 pp illus £15 00

W J W Sharrard's new comprehensive textbook on paediatric orthopaedics and fractures (21 chapters and 1 176 pages) compiles complete coverage of problems bearing on orthopaedic surgery and fractures in children It will therefore be welcomed not only by surgeons but also by paediatricians interested in this field Much space is justly given to congenital malformations and their treatment Sharrard is the official author but many colleagues have placed material at his disposal as is apparent from the acknowledgements

Sharrard's book also covers such diseases as tuberculosis and poliomyelitis which though they have now become less important in Europe and USA still play a substantial role in the panorama of diseases in other parts of the world Remarkably good photographic reproductions in every chapter illustrate amply the symptomatology and as well the conservative and surgical treatment of the diseases discussed A comprehensive list of references is also given at the end of each chapter To my knowledge no other book available gives such a good and exhaustive description of the symptomatology and differential diagnosis of orthopaedic diseases as well as of problems encountered in their treatment both conservative and surgical The work is less suitable as a textbook for consecutive reading but excellent as a reference for all those whose occupations involve any aspect of paediatric orthopaedics or fractures in children Sharrard has devoted 100 pages to his favourite subject congenital and developmental abnormalities of the acetabulum For the sake of proportion this chapter should have been shortened For comparison it might be mentioned that 120 pages are devoted to fractures and joint injuries but no objections can be raised against the size of this chapter because treatment of traumatic injuries in children is mainly conservative Fig 108 on page 155 is given as an illustration of a so-called false positive von Rosen sign I think there is no risk of erroneously concerning the right hip as luxated in the child in question The direction of the neck clearly shows that the head is well within the acetabulum Apart from these minor remarks the work is excellent in every respect and should be a welcome newcomer to the library at every department of orthopaedics

Anders Hirsch

B Tennant (ed) *Neonatal enteric infections caused by Escherichia coli* Ann NY Acad Sci vol 176 The New York Academy of Sciences New York 1971 405 pp US $28 00

The understanding of diarrhoeal disease in infancy has improved considerably during the last decade especially with regard to etiology pathogenesis and immunity This progress was the subject of a three day multidisciplinary conference during the spring 1970 sponsored and published by the New York Academy of Sciences Diarrhoea is common all over the world with a predeliction for underdeveloped societies where the climate is hot or cold In a primitive rural society diarrhoea was shown to reach its peak incidence during the latter half of the second year of life Although the infants were continuously exposed to EEC (enteropathogenic Escherichia coli) and Shigella these bacteria caused only 2 per cent of the diarrhoeas appearing below the age of six months as compared with 40 per cent during the second half of the second year The earlier the weaning period the earlier appeared the diarrhoeal peak In urban communities the peak incidence of diarrhoea appeared already during the first year of life In western societies EEC induced diarrhoea is a disease mainly of the newborn period Two explanations are offered for these differences an almost 100 per cent breast

feeding in the primitive society as opposed to a much lower incidence in urban areas and in western communities and—probably of equal importance—the stable eco system of the family in the primitive rural society as compared with the unphysiologic aggregation of neonates in hospital accompanied by exposure to strange E. coli strains

Intubation studies have shown that diarrhoea is a disease of the small intestines E. coli causing diarrhoea differ from other E coli in three respects they produce an enterotoxin are able to colonize the small intestine and to penetrat cells The reason for the two later effects is unknown The production of enterotoxin which causes movement of water and electrolytes over the gut epithelium is governed by plasmides (extra chromosomal DNA) The plasmides might be transmitted to different bacteria giving them enterotoxic properties Enterotoxicity is thus not bound to EEC but their common association with diarrhoeas might be explained by the fact that they are good receptors for plasmides

About 20 per cent of diarrhoea is caused by Shigella EEC and Salmonella in this order of frequency The etiology is unknown in the remaining 80 per cent By searching enterotoxin producing *E coli* rather than EEC strains it has now been possible to identify the etiologic agent in no less than 50 per cent of diarrhoeas of so called unknown origin Such advances will improve considerably epidemiologic studi s of diarrhoea

Recent studies suggest that IgA copro antibodies may participate in the local defence of the gut against EEC although the mode of action is unknown During early life specific IgA antibodies are furnished by breast milk later probably locally produced antibodies appear Recently immunization by the oral route has been attempted with promising results This book brings knowledge about a common sometimes life threatening and always unpleasant dis ase and can be recommended for all interested in th field

Jan Winberg

Mette Warburg *Diagnosis of metabolic eye diseases* Munksgaard Copenhagen 1972 112 pp Dkr 115—

Dr Warburg who is a consultant ophthalmologist to the Danish institutions for the mentally retarded has in her book collected some seventy five diseases related to inborn errors of metabolism in which an abnormal finding in eye structure or function can be of clinical value

The book is arranged so that the primary keyword relates to the topography of the opthalmological features There are sections dealing with the eye as a whole with the eyelid conjunctiva cornea etc Beneath the ophthalmological keywords we find the keywords of the metabolic disorder involved then the final diagnosis and after this a brief summary with the ophthalmological findings the general remarks of symptomatology metabolism different findings from the laboratory studies genetics and treatment if it is available After each disorder there are a few references to important and recent literature

The book is primarily designed for ophthalmologists but for others dealing with children with metabolic disorders and with children showing a more or less distinct symptom pattern with an eye abnormality this is a very helpful book to reduce the number of possibilities

As the purpose of the book is to help in the recognition of a number of these disorders early and to give easy access to further examination and to the literature there are no pictures and the book as a whole takes the form of a manual

It can be recommended to all paediatricians working in paediatric neurology and/or inborn errors of metabolism and to all those who deal with patients with mental retardation cerebral palsy and many other diseases There are perhaps too few cross references so that it is sometimes necessary to use the subject index but on the other hand the index is so extensive that this is a minor disadvantage

J C Melchior

ANNOUNCEMENTS

XVII CZECHOSLOVAK PEDIATRIC CONGRESS WITH INTERNATIONAL PARTICIPATION

will be held in Bratislava from September 5th to 7th 1973

Topics

1 Physiology of Immunity in Children
Pathology of Immunity
Autoimmunoaggressive Diseases
Immunodeficient States
Allergic Disease in Children

3 Latest Diagnostic and Therapeutic Notions

Further information by the secretary general doctor Mikulas Rusnak Slovenska Lekarska Spolocnost Mickiewiczova 18 Bratislava Czechoslovakia

ANNUAL MEETINGS OF THE AMERICAN PEDIATRIC SOCIETY THE SOCIETY FOR PEDIATRIC RESEARCH AND THE AMBULATORY PEDIATRIC ASSOCIATION

The annual meetings of the American Pediatric Society the Society for Pediatric Research and the Ambulatory Pediatric Association will be held in the San Francisco Hilton Hotel May 16–19 1973 The schedule for these meetings is as follows

Wednesday May 16 (a m and p m) Ambulatory Pediatric Association

Thursday May 17 (a m) Ambulatory Pediatric Association

Thursday May 17 (a m and p m) American Pediatric Society and Society for Pediatric Research subspecialty sessions

Friday May 18 (a m and p m) American Pediatric Society plenary sessions

Saturday May 19 (a m) Society for Pediatric Research plenary session (p m) American Pediatric Society and Society for Pediatric Research subspecialty sessions

For additional information write to Charles D Cook M D (Secretary American Pediatric Society 333 Cedar Street New Haven Connecticut 06510) Robert E Greenberg M D (Secretary Society for Pediatric Research 12012 Compton Avenue Los Angeles California 90059) or Elizabeth S Hillman M D (Secretary Ambulatory Pediatric Association 2300 Tupper Street Montreal Quebec Canada)

feeding in the primitive society as opposed to a much lower incidence in urban areas and in western communities and—probably of equal importance—the stable eco system of the family in the primitive rural society as compared with the unphysiologic segregation of neonates in hospital accompanied by exposure to strange *E coli* strains

Intubation studies have shown that diarrhoea is a disease of the small intestines *E coli* causing diarrhoea differ from other *E coli* in three respects they produce an enterotoxin are able to colonize the small intestine and to penetrate cells The reason for the two later effects is unknown The production of enterotoxin which causes movement of water and electrolytes over the gut epithelium is governed by plasmides (extra chromosomal DNA) The plasmides might be transmitted to different bacteria giving them enterotoxic properties Enterotoxicity is thus not bound to EEC but their common association with diarrhoeas might be explained by the fact that they are good receptors for plasmides

About 20 per cent of diarrhoea is caused by Shigella EEC and Salmonella in this order of frequency The etiology is unknown in the remaining 80 per cent By searching enterotoxin producing *E coli* rather than EEC strains it has now been possible to identify the etiologic agent in no less than 50 per cent of diarrhoeas of so called unknown origin Such advances will improve considerably epidemiologic studies of diarrhoea

Recent studies suggest that IgA copro antibodies may participate in the local defence of the gut against EEC although the mode of action is unknown During early life specific IgA antibodies are furnished by breast milk later probably locally produced antibodies appear Recently immunization by the oral route has been attempted with promising results This book brings knowledge about a common sometimes life threatening and always unpleasant disease and can be recommended for all interested in the field

Jan Winberg

Mette Warburg *Diagnosis of metabolic eye diseases* Munksgaard Copenhagen 1972 112 pp Dkr 115—

Dr Warburg who is a consultant ophthalmologist to the Danish institutions for the mentally retarded has in her book collected some seventy five diseases related to inborn errors of metabolism in which an abnormal finding in eye structure or function can be of clinical value

The book is arranged so that the primary keyword relates to the topography of the opthalmological features There are sections dealing with the eye as a whole with the eyelid conjunctiva cornea etc Beneath the ophthalmological keywords we find the keywords of the metabolic disorder involved then the final diagnosis and after this a brief summary with the ophthalmological findings the general remarks of symptomatology metabolism different findings from the laboratory studies genetics and treatment if it is available After each disorder there are a few references to important and recent literature

The book is primarily designed for ophthalmologists but for others dealing with children with metabolic disorders and with children showing a more or less distinct symptom pattern with an eye abnormality this is a very helpful book to reduce the number of possibilities

As the purpose of the book is to help in the recognition of a number of these disorders easily and to give easy access to further examination and to the literature there are no pictures and the book as a whole takes the form of a manual

It can be recommended to all paediatricians working in paediatric neurology and/or inborn errors of metabolism and to all those who deal with patients with mental retardation cerebral palsy and many other diseases There are perhaps too few cross references so that it is sometimes necessary to use the subject index but on the other hand the index is so extensive that this is a minor disadvantage

J C Melchior

Acta Paediat Scand 62 117–120 1973

PERINATAL MORTALITY IN ICELAND

GUNNAR BIERING

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Perinatal mortality is the most valid statistical expression of quality of newborn care and hence enables comparisons to be made with results obtained elsewhere The data presented are the first of such statistics to be presented from Iceland

In Iceland the Public Health Reports (Heilbrigðisskýrslur) supply information concerning only infant mortality Stillbirths are also registered however deaths during the first seven days after birth are not recorded Accordingly health authorities in Iceland do not report perinatal mortality

Iceland has not yet adopted the WHO definitions on viability presented in 1950 The viability criteria in use date back to a regulation issued in 1933 and differ from the WHO definitions on vital points Of main interest is the fact that a non breathing infant at birth is considered stillborn in spite of the presence of other signs of life such as heartbeat and movements of voluntary muscles The viability criteria in Iceland are presently under revision and will be coordinated with new regulations to be issued by WHO in 1975

In collecting data on perinatal mortality in Iceland the WHO definitions were applied except that newborns weighing 600 g and less were excluded even if they exhibited signs of life such as gasping heartbeat and movements of voluntary muscles

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This is the first paper of a series dealing with paediatric problems in the Northern parts of the Scandinavian countries

PERINATAL MORTALITY IN REYKJAVIK 1961–1970

Data on perinatal mortality in Reykjavik were collected and published in 1971 (3) During this period 23 502 infants were born in Reykjavik approximately half of the total newborn population in Iceland during the decade Table 1 shows the newborn population in the whole of Iceland during the same period

Fig 1 shows perinatal mortality in Reykjavik 1961–1970 Due to the limited case material and the resulting fluctuations in the mortality figures from year to year the 10 year period was divided into two 5 year periods to secure a more solid basis for comparison (Table 2) The difference in perinatal mortality shown in the table is not statistically significant

In spite of the fact that approximately half of the newborn population in Iceland derives

Table 1 *The newborn population in Iceland 1961–1970*

	No of infants
1961	4 563
1962	4 711
1963	4 891
1964	4 845
1965	4 692
1966	4 749
1967	4 454
1968	4 225
1969	4 200
1970	4 018

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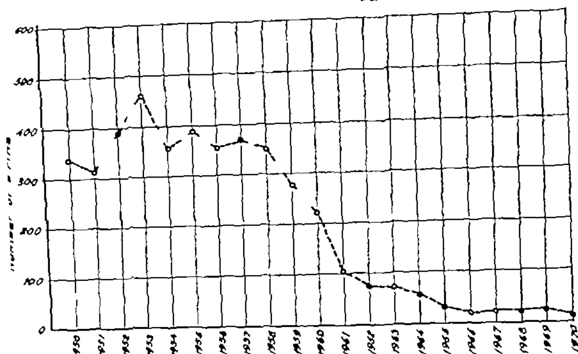


Fig 2 Home deliveries in Reykjavik 1950–1970

DEVELOPMENTAL PLANS

Iceland recently joined a WHO research project which included a trial of a new certificate for the *outcome of* pregnancy At the same time previous records of pregnancy and its outcome *in* the *country* were revised The reporting of all deaths during the first seven days *of life* has been made obligatory among other changes and this will make data collecting concerning perinatal mortality possible on a year *to* year basis

Furthermore new maternity records have been designed and distributed throughout the country to be used for all obstetrical cases replacing individual records used in various hospitals It is hoped that these measures will be of help in coordinating obstetrical and neonatal care in Iceland in the future

Table 4 *Deliveries in Iceland and Reykjavik 1970*

	Iceland	Reykjavik
No of deliveries	3 986	2 186
Twins	31	16
Triplets	1	1
Males	2 073	1 154
Females	1 945	1 050
Total no of newborn infants	4 018	2 204
Perinatal mortality	18.2/1 000	17 0/1 000
Stillbirths	10 0/1 000	9 0/1 000
Deaths during first week	8.2/1 000	8 0/1 000
Premature (<2 500 g)	150–3 7	96–4 4
Perinatal mortality	4 7	5 0
Stillbirths	13 3	13 5
Deaths during first week	11 4	11.5

SUMMARY

Data concerning perinatal mortality in Iceland in *1970* are presented

The total number of newborn infants was 4 018 Perinatal mortality was 18 3/1 000

Information concerning perinatal mortality in Reykjavik 1961–1970 is also presented and comparisons made between Reykjavik and the country at large

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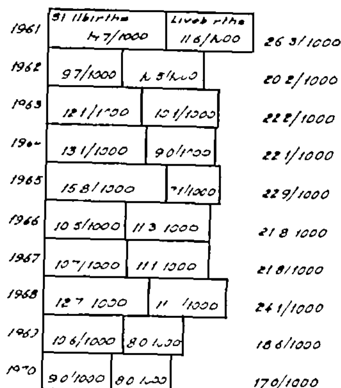


Fig 1 Perinatal mortality in Reykjavik 1961–1970

from Reykjavik it was felt that the data from Reykjavik might not represent the country at large The fact that Landspitalinn i Reykjavik (the University Hospital) is the only large hospital in the country with adequate facilities for obstetrical and perinatal care is of interest in as much as that hospital deals with the majority of abnormal obstetrical cases from the entire country One could for example point out a premature rate of 7 0% within

Table 2 *Deliveries in Reykjavik 1961–1970*

	1961–1965	1966–1970
Number of deliveries	11 842	11 373
Twins	158	105
Triplets	2	4
Males	6 120	5 926
Females	5 882	5 560
Total number of newborn infants	12 002	11 486
Perinatal mortality	22 7/1 000	20 7/1 000
Stillbirths	13 0/1 000	10 7/1 000
Deaths during first week	9 7/1 000	10 1/1 000
Premature (≤2 500 g)	4 3	4 6
Perinatal mortality	29 9	25 5

Table 3 *The distribution of hospital deliveries in Iceland 1970*

No of deliveries	No of hospitals
More than 100	8
20–100	11
Less than 20	6

that hospital in 1970 while the Reykjavik rate was 4 4% and that of Iceland 3 7% Also approximately 40% of all obstetrical admissions to the hospital were non municipal

PERINATAL MORTALITY IN ICELAND 1970

Nationwide perinatal data for Iceland were collected to obtain a clearer picture of the perinatal mortality in the country at large As mentioned earlier the WHO viability definitions were adopted in collecting these data

The total number of infants delivered was 4 018 The distribution of hospital deliveries thoughout the country is shown in Table 3 210 infants were delivered at home which constitutes 5 2% of the total number of deliveries in the country Home deliveries have rapidly decreased in number during the past 20 years (Fig 2)

It is assumed that the number of home deliveries will decline further and in the future approximately 20 hospitals in the country are expected to become adequately equipped for routine obstetrical care Obstetrical and neonatal complications will be dealt with to an increasing extent by the major centers of Reykjavik and Akureyri To further this aim a closer contact between the major centers and the peripheral hospitals will be developed systematically

Table 4 shows the pertinent perinatal data in Iceland and Reykjavik in 1970

It is of interest how slight the difference in perinatal mortality is between Reykjavik and the country at large particularly the fact that the mortality is lower in Reykjavik

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THE INFLUENCE OF THE PLACENTAL TRANSFUSION ON THE CAPILLARY BLOOD GAS AND ACID–BASE BALANCE IN THE NEWBORN INFANT

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Where clamping of the umbilical cord is delayed for a few minutes about 100 ml of blood is transferred from the placenta to the newborn (3) and the acid–base balance of the latter is affected Thus it has been demonstrated (7) that the arterial P_{co} during the first 3 hours of life is higher in late clamped newborns than in early clamped while the standard bicarbonate of the latter is lower

As the use of capillary blood has become increasingly common for measuring pH P_{co} and standard bicarbonate in newborns we have investigated the influence of the placental transfusion on the acid–base balance of the capillary blood during the first day of life Three to four hours after birth we have found an increased metabolic acidosis in late clamped normal newborns as compared with early clamped This finding will be discussed in relation to the postnatal transudation of fluid into the capillary beds

Earlier Investigations

From Table I it appears that pH P_{co} and standard bicarbonate with a single exception (2) are identical in early and late-clamped newborns when measured on capillary blood 1–3 and 24 hours after birth It also appears that P_{co} and standard bicarbonate generally are higher in the capillary blood than in arterial while pH is lower Finally it is seen that the acidosis found immediately after birth tends to decrease during the first day of life

MATERIAL AND METHODS

The investigation has been carried out on normal full term babies whose neonatal course was uneventful As a routine late clamping is applied to such newborns the upper airways being cleaned and the babies examined while the cord is still intact In 35 cases this procedure was maintained and this group was described as being late-clamped In 4 cases the cord was clamped as fast as possible i e within 15 seconds and this group was described as being early clamped With regard to sex, gestational age Apgar score and birth weight, the two groups did not differ just as they did not differ with regard to complications due to pregnancy and delivery With one exception all babies were delivered via vaginal route

pH Pco standard bicarbonate and hematocrit were measured 1 2 3–5 6–12 and 13– 4 hours after birth To avoid too many heel punctures only two samples were taken from each newborn We aimed to get about ten values for each time interval Furthermore we obtained placental blood from the umbilical cord in 10 late and 12 early-clamped babies

Capillary blood obtained by heel puncture of an unwarmed heel was collected in heparinized microtubes and examined immediately Actual pH and pH following equilibration with gas mixtures with known content of CO were measured using Radiometer apparatus (Model BM 2) for acid–base determination the apparatus being thermostated at 37 C Pco and standard bicarbonate were calculated from a nomogram specified for this equipment (9) and no correction was done for oxygen saturation and body temperature All determinations were made in duplicate the difference in pH not exceeding 0 009 units Hematocrit was measured after spinning the microcapillary tubes for 3 minutes

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3 Biering G Dánartolur nýfæddra barna í Reykjavík 1961–1970 (Perinatal mortality in Reykjavik 1961–1970) *Læknablaðið* 57 Vol 4 121 1971

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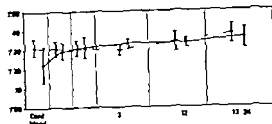


Fig 2 Actual pH in the capillary blood of 42 early clamped (●) and 35 late-clamped (O) infants during the first day of life Mean values ± S D

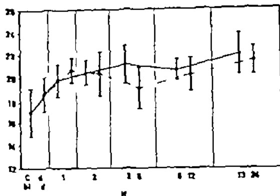


Fig 4 Standard bicarbonate (mEq/l) in the capillary blood of 42 early-clamped (●) and 35 late-clamped (O) infants during the first day of life Mean values ± S D

tions we believe that our findings confirm the above mentioned investigations

Regarding the acid–base balance of the capillary blood our results as a whole confirm the results of others (4 6) Thus the acid–base balance of the capillary blood was unaffected by the placental transfusion when measured 1–2 and 6–24 hours after birth

Comparing our results with those obtained by using arterial blood samples (7) we revealed a discrepancy also found by others (5) By using capillary blood P_{CO} and standard bicarbonate seem to be estimated too high while actual pH is measured too low The reason for this difference has been thought to be related to poor peripheral circulation either due to cooling or delayed closure of the ductus arteriosus (5) but in our opinion the placental transfusion may be one of the factors responsible at least when measurements are performed at 3 to 5 hours of age While the clinical condition of early and late-clamped newborns appeared to be identical and normal we found thus at this time a lower pH and a lower standard bicarbonate in late-clamped babies Since there is a negative correlation between actual pH and hematocrit the *difference* in actual pH may partly be caused by the transcapillary fluid transudation which follows the placental transfusion This finding supports the concept that the acid–base status of capillary blood is unreliable when oedema is present (1)

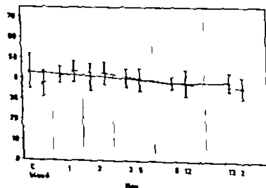


Fig 3 P_{CO} (mmHg) in the capillary blood of 42 early-clamped (●) and 35 late-clamped (O) infants during the first day of life Mean values ± S D

SUMMARY

Of 77 normal newborns 42 were clamped early while 35 were clamped late Using capillary *blood from* an unwarmed heel the hematocrit and the acid–base balance of the two groups were compared During the first day of life the hematocrit of late-clamped newborns was fairly constant and higher than that of early clamped babies the latter showing a definite fall 2–24 hours after birth Regarding the acid–base balance no difference was found between the two groups of newborns except when measurements were *performed 3–5 hours* after birth At this time actual pH and standard bicarbonate were lower in late-clamped babies than in early-clamped while P_{CO} was

Table 1 *Mean values of pH P_{co} (mmHg) and standard bicarbonate (mEq/l) in the blood of early and late clamped normal newborns during the first day of life*

EC – early clamped LC – late clamped + – significant difference P – present series

Ref	pH		P_{co}		Standard bicarbonate		Age	Blood
	EC	LC	EC	LC	EC	LC	(hours)	
(7)	7 24	7 30+	55	45	18 9	20 1+	1/2	Capillary
(6)	7 29	7 31	45	47	19 6	20 8	1/2	Capillary
(4)	7 25	7 28					1/2	Capillary
(7)	7 35	7 37	27	30	17 4	19 7	1/2–1	Arterial
(6)	7 34	7 32	43	45	21 5	21 0	1	Capillary
P	7 29	7 30	42	44	19 8	20 6	1	Capillary
(7)	7 36	7 35	27	31	17 3	18 5	1–3	Arterial
(6)	7 37	7 36	41	42	22 4	22 2	2	Capillary
P	7 32	7 31	42	43	20 4	20 3	2	Capillary
(4)	7 34	7 34					3	Capillary
P	7 33	7 30+	40	40	21 1	19 0	3–5	Capillary
(7)	7 37	7 35	30	30	18 3	18 0	3–5	Arterial
P	7 33	7 34	39	39	20 6	20 2	6–12	Capillary
(7)	7 40	7 37	28	28	19 0	18 4	5–24	Arterial
P	7 36	7 38	39	36	22 0	21 4	13–24	Capillary
(4)	7 39	7 39					24	Capillary

RESULTS

From Fig 1 it appears that hematocrit values measured on cord blood were identical in early and late-clamped newborns During the first 2 hours the hematocrit rose in both groups the highest levels being reached in late clamped babies From 3–24 hours of life the hematocrit of late-clamped babies was fairly constant and still higher than that of early clamped babies the latter showing a moderate fall

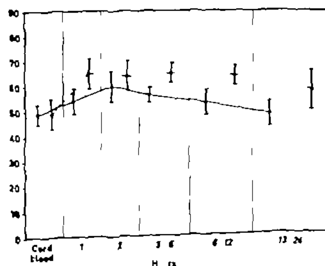


Fig 1 Hematocrit of the capillary blood of 42 early clamped infants (●) and 35 late-clamped infants (O) during the first day of life Mean values ±S D

From Figs 2 3 and 4 it appear that pH P_{co}, and standard bicarbonate were little affected by the techniques used for clamping of the cord However an exception was found *during the period between 3 and 5 hours* after birth where pH and standard bicarbonate were significantly lower among late-clamped newborns than in early clamped ($p<0\ 05$ using the non parametric test of White)

At 3–5 hours of age a significant correlation was demonstrated between actual pH and capillary hematocrit actual pH = 7 32–0 00276 × (hematocrit – 60 3) correlation coefficient = – 0 488 $p<0\ 05$

DISCUSSION AND CONCLUSION

An investigation very similar to ours has established that the capillary hematocrit of late clamped babies rises during the first hours of life probably due to transcapillary loss of fluid into the interstitial space (8) It has also been demonstrated (8) that in early-clamped babies a moderate fall in hematocrit *during the first* day of life occurred, probably due to absorption of fluid from the interstitial space Although we did not carry out serial determina

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A LONG TERM FOLLOW UP INVESTIGATION OF PATIENTS WITH HYPERTROPHIC PYLORIC STENOSIS—WITH SPECIAL REFERENCE TO THE PHYSICAL AND MENTAL DEVELOPMENT

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The adverse effect of malnutrition in infancy for later development, physical as well as mental, has been emphasized in a number of reports (5, 14). Investigations have been made in populations with a low socio-economic background (9) as well as among groups who have moved to countries with better socio economic conditions, such as Japanese children in USA (10), Puerto Rican boys in New York (1) and northern Lapps moving further south in Sweden (17).

Common factors for all of these investigations are that the malnutrition during the period of development of the child had a duration far beyond infancy and that many other negative factors due to the poor socio-economic background had had influence, with the spectrum of damage reaching from repeated infections to lack of intellectual stimulation.

Thus it is difficult to distinguish the result of malnutrition per se in humans. In experimental animals the effects of early malnutrition is easier to study (12). In rats the final size of the animal has been demonstrated to be dependent on the caloric intake during the weaning period, an extra caloric supply after starvation during weaning had no effect on the small final size of the animal (11).

Even more difficult to evaluate is the effect of inanition on the mental development, but the findings of Vahlqvist et al that inanition in humans diminishes the size of the brain as measured by echoencephalography (23) indicate that inanition might give a reduction of mental capacity. This finding is also in accord with the profound effect of neuronal growth of early inanition in animals demonstrated by quantitative cytochemical methods (13). In an attempt to determine the effect of early malnutrition in humans a follow up study has been done on a number of Swedish boys who suffered from hypertrophic pyloric stenosis. The disease in these patients started between the ages of 6 and 20 days, after which time the inanition began and had a varying duration. With recovery their caloric intake normalized and their opportunity for food intake was the same as that for randomly selected Swedish children.

MATERIAL

During the period 1922 through 1942 203 boys were treated for hypertrophic pyloric stenosis at the Children's Hospital, Gothenburg (Head Prof A Wallgren). They were born at home or at different maternity hospitals in western Sweden, the main part however came from the City of Gothenburg. The diagnosis was confirmed by X ray except for a few patients in 1922 and 1923. All patients had the same type of treatment, numerous small feedings by spoon, spasmolytics and in some cases subcutaneous salt solution with glucose. None was operated on.

One case history of the 203 is missing. It concerned a boy who died one year later of a kidney malformation. Investigation and follow up of the other 202 records showed that 2 children emigrated

identical On the basis of the hematocrit findings it is proposed that the placental transfusion by creating oedema of the interstitial space may contribute to the well known discrepancy between the acid–base status of the capillary and the arterial blood of the newborn

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Table 1 *The mean values of some parameters in the different groups*

	Group I	Group II	Group III	Group IV
Number	56	57	44	23
Birth weight (g)	*3 420±511*	*3 560±330*	*3 800±492*	*3 790±546*
Weight deficit (%)	21.0±9.7	35.5±8.3	35.3±4.9	47.1±7.5
Duration (weeks)	—	4.9	9.7	14.8
Adult height (cm)	175.9±6.3	177.2±6.5	176.1±6.5	173.3±6.9
Intelligence test	5.1 (47/56)	5.5 (49/57)	5.0 (38/44)	4.8 (18/23)
Adaptation test	4.5 (19/56)	4.6 (25/57)	4.1 (16/44)	3.9 (7/23)
Fertility (% with children)	66.1	62.5	70.5	45.5

Mean values ±S D

comparison with the mean height of Swedish recruits The height at the time of the *interview was compared* with that of brothers

To estimate the mental development of the patients the results of the intelligence test at registration for military service were used This test has been given *to all military recruits since 1943* so these data are lacking in patients born before 1925 The results of this test for each year are adjusted *to a mean value* of 5.0 with a distribution from 1 = low to 9 = high intelligence rating according to a Gaussian curve Most of the recruits with a score of 5 or more were given a special adaption test constructed by the military psychological team in which adjustment to work and society and emotional stability were rated The scoring was according *to the same scale* as the intelligence test

RESULTS

The average birth weight was 3 420 g in group I 3 560 g in group II 3 800 g in group III and 3 790 g in group IV (Table 1) These values and the average course of the disease that is the mean minimum weight and the time of it are presented for each group in Fig 3 The *high birth weight* of children developing hyper trophic pyloric stenosis is also earlier demon strated (6)

The means of adult height for the *groups* decreased with increasing severity of the mani tion i e the mean for *group II* was 177 2 cm for group III 176 1 cm for group IV 173 3 cm and for the *mixed* group 175 9 cm The average height of the Swedish recruits born *between* 1922 and 1942 was 176 8 cm (4) This numerically lower height in the group of more severe malnutrition during infancy has been tested by means of multiple linear *regression* analysis with the adult height as the dependent factor and various variables from the initial period such as birth weight age of onset age of minimum weight weight deficit and dura tion as the *contributory factors* *Because* the birth weight increased in the group with the more severe malnutrition partial correlation was counted At a constant birth weight the partial correlation was negative on a 1 level between the weight deficit and the adult height In those belonging to group II–IV the *duration of the disease* was calculated and there the partial correlation was also negative on a significant level between the duration and the adult height

No significant correlation could be obtained using the other *parameters* *The effect of the* inanition was also demonstrated by comparing the adult height of the former patients with *that of their brothers* *Of 88* former patients with brothers only 15 were taller than their *brothers* (Table 2) The intelligence test as well as the adaptability test demonstrated a

Table 2 *Adult height of patients with hypertrophic pyloric stenosis compared with that of their brothers*

Group	Number with brothers	Brothers taller	Brothers same height	Brothers shorter
I	28	11	10	7
II	29	14	9	6
III	22	16	5	1
IV	9	5	3	1
Total	88	46	27	15

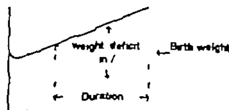


Fig 1 Schematic drawing for explaining how weight deficit and duration of the disease is calculated

fairly soon after recovery Three others were only temporarily in Gothenburg and not registered in the parish records Another boy became a sailor and has not been in the country for the last 15 years The whereabouts of a fifth presently 40 years of age known to have had epileptic seizures is unknown Fifteen patients died during childhood The morbidity and mortality among the patients are presented in another paper (2) Thus at the time for the military service 180 were available but of them three died and one emigrated before the time of the interview To determine the effect of the poor caloric intake in infancy the material was divided in four groups in regard to the severity of the inanition To evaluate this the weight deficit at the time the child attained his minimum weight was calculated in per cent of the predicted weight that the child ought to have had at that time based on his birth weight according to von Sydow's investigations of weight gain of Swedish children in 1930s (22) The duration of the disease was counted from the onset until the time the patient regained his birth weight (Fig 1) The values are plotted in Fig 2 with the time in weeks on the X axis and the weight deficit in per cent on the Y axis There is a correlation between duration and weight deficit and on this basis the patients were arbitrarily divided into three groups group II (57 patients) with a slight inanition of a short duration and moderate weight loss group III (44 patients) with a moderate inanition and group IV (23 patients) with a severe inanition Group I with 56 patients is a mixed group It comprises patients whose weight never dropped below the birth weight so the duration of the disease could not be calculated Though a few in this group had suffered a severe weight loss, the main part seemed to have had a fairly slight inanition

METHODS

At the interview were noted the height and weight of the patients the height of their brothers, the education and jobs of the patients and their siblings

The height of the individual on beginning military service was procured from the military records for

I 175.8 cm II 177.2 cm III 178.1 cm IV 173.3 cm

duration in weeks; weight deficit in %

Fig 2 The group division of the patients according to the duration of the disease and the weight deficit The duration of the disease could not be calculated in group I

Table 1 *The mean values of some parameters in the different groups*

	Group I	Group II	Group III	Group IV
Number	56	57	44	23
Birth weight (g)	3 470±511	3 560±530	3 800±492	3 790±546
Weight deficit ( )	21 0±9 7	35 5±8 3	35 3±4 9	47 1±7 5
Duration (weeks)	—	4 9	9 7	14 8
Adult height (cm)*	175 9±6 3	177 2±6 5	176 1±6 5	173 3±6 9
Intelligence test	5 1 (47/56)	5 5 (49/57)	5 0 (38/44)	4 8 (18/23)
Adaptation test	4 5 (19/56)	4 6 (25/57)	4 1 (16/44)	3 9 (7/23)
Fertility (with children)	66 1	62 5	70 5	45 5

Mean values ±S D

comparison with the mean height of Swedish recruits The height at the time of the interview was compared with that of brothers

To estimate the mental development of the patients the results of the intelligence test at registration for military service were used This test has been given to all military recruits since 1943 so these data are lacking in patients born before 1925 The results of this test for each year are adjusted to a mean value of 5 0 with a distribution from 1 – low to 9 – high intelligence rating according to a Gaussian curve Most of the recruits with a score of 5 or more were given a special adaption test constructed by the military psychological team at which adjustment to work and society and emotional stability were rated The scoring was according to the same scale as the intelligence test

RESULTS

The average birth weight was 3 420 g in group I 3 560 g in group II 3 800 g in group III and 3 790 g in group IV (Table 1) These values and the average course of the disease that is the mean minimum weight and the time of it are presented for each group in Fig 3 The high birth weight of children developing hypertrophic pyloric stenosis is also earlier demonstrated (6)

The means of adult height for the groups decreased with increasing severity of the inanition i e the mean for group II was 177 2 cm for group III 176 1 cm for group IV 173 3 cm and for the mixed group 175 9 cm The average height of the Swedish recruits born between 1922 and 1947 was 176 8 cm (4) This numerically lower height in the group of more severe malnutrition during infancy has been tested by means of multiple linear regression analysis with the adult height as the dependent factor and various variables from the initial period such as birth weight age of onset age of minimum weight weight deficit and duration as the contributory factors Because the birth weight increased in the group with the more severe malnutrition partial correlation was counted At a constant birth weight the partial correlation was negative on a 1 level between the weight deficit and the adult height In those belonging to group II–IV the duration of the disease was calculated and there the partial correlation was also negative on a significant level between the duration and the adult height

No significant correlation could be obtained using the other parameters The effect of the inanition was also demonstrated by comparing the adult height of the former patients with that of their brothers Of 88 former patients with brothers only 15 were taller than their brothers (Table 2) The intelligence test as well as the adaptability test demonstrated a

Table 2 *Adult height of patients with hypertrophic pyloric stenosis compared with that of their brothers*

Group	Number with brothers	Brothers taller	Brothers same height	Brothers shorter
I	28	11	10	7
II	29	14	9	6
III	22	16	5	1
IV	9	5	3	1
Total	88	46	27	15

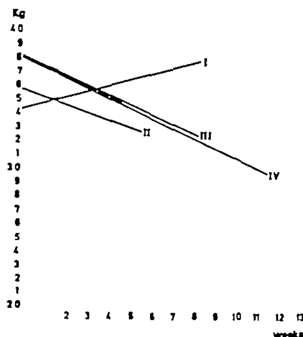


Fig 3 The average course of the disease in the different groups the birth weight the minimum weight and the time of the minimum weight

slight decrease in the numeric values with increasing malnutrition groups II–IV (Table 1) but the differences were not statistically significant In the recent interview these men at that time 27 to 47 years of age had a low fertility 36 were married, but without children In total there were 189 children 118 of whom were boys Group IV had the lowest fertility (Table 1)

Socially the former patients were fairly well adapted at the time of the interview All of those interviewed had work except two imbeciles one belonging to group II and the other to group IV and one blind man belonging to group II

DISCUSSION

A limited time of inanition in early infancy affects statistically significantly the final size of the individual which will be correlated both to the duration of the inanition and the weight deficit This is not in accord with the findings of Steinecke (20) and Rinvik (19) but if the greater birth weight of the children with hypertrophic pyloric stenosis is not taken in account the differences of the final size of those with more severe inanition from the normal population will be only numerical

A numerical though not statistical difference was found in the intelligence test between the group with more severe inanition and the normal population This might be considered that the mental development is somewhat more resistent against inanition than the growth at least if the inanition lasts only for a limited period after birth It might be that in humans the period of greatest risk for disturbance of mental and perhaps also physical development by a limited period of inanition is already passed at the time of delivery something which has been stressed by some reports (7 15 18)

The tendency demonstrated by the result of the adaptability test is in accord with the findings of Stock & Smythe (21) who in their investigation of twenty undernurished Cape infants found that they between the age of five to ten years differed significantly from their control group in the performance of the intelligence test, but that they also scored significant lower in the test of motivation and in the personality variables as initiative and fantasy affectionate behaviour

A somewhat unexpected finding was the low fertility in the group with severe inanition as well as that the sons outnumbered the daughters two fold Similar findings have not been reported In Carter s large material (3) of more than 700 patients the distribution of the children according to sex was equal His patients had been operated upon and had thus not suffered from prolonged malnutrition It might be that inanition early in life might affect reproductive capacity This seems not to be in accord with the overpopulation in many of the underdeveloped countries but there the malnutrition for those who are adult today usually started later after the weaning period

SUMMARY

202 of 203 patients medically treated for hypertrophic pyloric stenosis at the Children s Hospital in Gothenburg 1922 to 1942 were classi

fied according to the severity of their malnutrition during their disease. In the 180 registered for military service height and the intelligence test results were investigated and 176 of these were recently interviewed.

A significant correlation was found between the adult height and the weight loss and duration of the malnutrition in infancy at a constant birth weight. A numerical but not statistically significant difference could be demonstrated in the intelligence and adaptability tests between the patients with more severe undernutrition and those of the same age at the time they entered military service.

Another finding was that undernutrition in infancy seemed to be associated with a decreased fertility in men, especially in the string of girls.

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Thanks are extended to Professor A. Wallgren for generously making his well studied material available, a material collected when he was head of Children's Hospital in Gothenburg. Without his encouraging help and interest this work would never have come into being.

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A LONG TERM FOLLOW UP INVESTIGATION OF PATIENTS WITH HYPERTROPHIC PYLORIC STENOSIS—WITH SPECIAL REFERENCE TO HEREDITY AND LATER MORBIDITY

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In a follow up study of former patients with hypertrophic pyloric stenosis (HPS) one of the main questions is if the former patient in later life will demonstrate increased mortality and morbidity but of almost the same interest is to judge the risk for his descendants to get the disease

MATERIAL

Between 1922 and 1942 203 boys were treated for hypertrophic pyloric stenosis at the Children's Hospital Gothenburg This number includes not only all the boys with this disease from the city of Gothenburg but also boys with hypertrophic pyloric stenosis from all of western Sweden who had the disease severely enough to require specialist treatment

The follow up study includes only 195 former patients because 8 patients could not be traced of these three are known to have emigrated as children All of the patients were treated medically with numerous small feedings spasmolytics and in some cases subcutaneous injections of saline or glucose solutions None was operated on The severity of the disease varied from cessation of vomiting and a steady weight gain from the day after hospitalization to a progressive disease lasting up to 22 weeks before any steady weight gain was obtained 79 of the 203 started their weight gain within three weeks while 32 did not show any weight gain for more than 12 weeks

RESULTS

Mortality

There was no mortality in the disease in contrast to other Scandinavian medically treated materials where the mortality in hypertrophic pyloric stenosis has been about 3° (8) though seven died during infancy Two of these died of pulmonary changes following aspiration when they had begun a steady weight gain at three and four months respectively Two others died at 4 months of age one of septicemia the other of capillary bronchitis Another who on admission to hospital was noted to have a generalized severe muscle hypertonia died of his muscular disease at 9 months of age One died at an orphanage with sudden death at 8 months of age One with a kidney malformation died of pyelitis and kidney insufficiency at 12 months of age Two more died of disease as toddlers one of meningitis at 14 months of age the other of pneumonia at 17 months of age During school years 3 more patients died one each in pneumonia leukaemia and influenza

During childhood two children drowned and two were killed in traffic accidents

After reaching adult age three died one of subarachnoidal bleeding one of a shot laceration and one had committed suicide—he also had diabetes The mortality among these patients during childhood should be compared with the average for that age group during the years 1922 to 1942 For infants after the neonatal period the mortality in Sweden decreased from 4 6 in 1922 to 1 5 in 1942 Among the patients with hypertrophic pyloric stenosis the mortality during the first year of

This work was supported by a grant from AB Semper's Fund of Nutritional Research

Table 1 *Morbidity in former patients with hypertrophic pyloric stenosis*

Disorder	Total
Peptic ulcer	13
Gastritis	23
Diabetes	3
Ulcerative colitis	1
Malignant tumour	2
Epileptic seizures	2
Alcoholism	2
Dorsal insufficiency	3
Nervous trouble	7

Ca prost 1 sarcoma 1

life was 3 6% which hardly deviates from the average

After infancy there does not seem to be any increased mortality in other diseases The high number of violent deaths however is surprising but might be related to the numeric though not statistically significant finding that former HPS patients were not quite as socially adjusted as the average individual (2)

Morbidity

176 of the former HPS patients were interviewed and their morbidity is presented in Table 1 Who were imbeciles one was a twin whose twin brother was healthy the other one had an imbecilic mother and brother One who had diabetes was blind All the others were employed at the time of interview however two had been on sick leave for more than a year because of ulcerative colitis and cancer prostata respectively Seven others reported sick leaves lasting for more than two months because of nervous troubles dorsal insufficiency and alcoholism

Table 2 *Incidence of hypertrophic pyloric stenosis in relatives of 176 male index patients*

	Brothers	Sisters	Sons	Daughters
Total	151	154	123	66
Affected	14	4	4	2
Incidence	1 11	1 39	1 31	1 33

Thirteen patients had had peptic ulcer the distribution did not seem to be correlated to the duration of the HPS because the 79 who recovered within three weeks had almost the same incidence as the rest of the former patients 23 had had dyspeptic complaints thirteen of those had had an X ray which did not demonstrate any ulcers in the rest the complaints had been so minimal that no examination had been performed No one complained of vomiting In the general population dyspeptic complaint is a more common disease than peptic ulcer so it was also among the former HPS patients The morbidity in peptic ulcer was 7 4 an incidence which seems not to be increased as compared to the general population where a survey of 50-year old men in Gothenburg shows an incidence of 16 with peptic ulcers (5) The mean age of the former HPS patients was 35 5 years The findings in this material that the former patients did not have an increased morbidity in peptic ulcer are not in accord with those of Steinecke (4) who pointed out that the treatment of HPS with repeated stomach washings may give rise to later gastric disorders Such a treatment had not been used in the present material Also such patients who have been operated for HPS have demonstrated a higher frequence of peptic ulcers later in life (1)

Heredity

To analyze the heredity pattern of HPS its presence in the siblings and children of the 176 interviewed former patients was sought 135 of the 176 had siblings in total 305 105 former patients had 189 children (Table 2)

One of the patients had two brothers with the disease and another had one sister and one brother with the disease The latter patient was married but without children

The heredity patterns demonstrated the greatest risk for the brothers of the patient an incidence of 1 11 Of the children the boys seemed to have the same risk as the girls an incidence of 1 31 compared with

1 33 The number are however too small for statistical work-up The figures are not in accord with the somewhat larger material of Carter (3) where with 281 former male patients the risk for the sons was 1 16 and that for the daugthers 1 39

He concluded from this material as well as from investigations of the heredity patterns of girls with the disease that HPS is the result of combined hereditary and environmental factors, where the hereditary factors have poly genic patterns That a hereditary factor is present is demonstrated by an incidence of HPS of 4 5% in close relatives of the pre sented material Wallgren demonstrated that in the population the incidence of the disease was 0 4% in 1930 but had decreased to 0 2% in 1960 (6 7)

SUMMARY

Of 203 boys treated for hypertrophic pyloric stenosis at Children s Hospital Gothenburg during the years 1922–1942 a total of 195 could be traced by a follow up investigation in 1970 All had been medically treated Seven died during their first year of life none of the dis ease directly This number is the approximate figure for infant mortality in Sweden at that time

The morbidity among the former patients did not differ from that for the general popula tion 7 5% had had peptic ulcer the mean age at the interview was 35 6 years This can be compared with a frequency of peptic ulcer at 16% for 50 year olds in the population in Gothenburg

4 5% of close relatives had had hypertrophic pyloric stenosis The risk for the sons and daugthers was about the same 1 30 The brothers were the ones with the greatest risk 1 11

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TREATMENT OF DIABETES INSIPIDUS IN CHILDREN WITH DDAVP A SYNTHETIC ANALOGUE OF VASOPRESSIN

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The management of vasopressin sensitive diabetes insipidus in children involves many therapeutical difficulties All aqueous vasopressin preparations at present commercially available have a short duration of action Irrespective of the mode of administration—intramuscular injection or intranasal insufflation—the effect of aqueous pitressin or synthetic 8 lysine vasopressin (LVP) lasts about 2–4 hours Pitressin tannate in oil (PTO) given intramuscularly is effective for 24 hours or more but the injections can be painful and the antidiuretic effect is sometimes variable and difficult to control Side effects such as headache and abdominal discomfort and the mode of administration sometimes make this preparation unpopular with both patients and parents Drugs with antidiuretic effect at present available for oral use (chlorpropamide thiazide diuretics clofibrate and carbamazepine) in many cases have disturbing side effects or fail to adequately control the urine production (4–6 8–10 15–17 19–22)

1-deamino 8 D arginine vasopressin DDAVP is a new synthetic analogue of vasopressin which lacks the pressor activity of pitressin and LVP and has a specific and long lasting antidiuretic action (1 23) In adults with diabetes insipidus it has been demonstrated to effectively control the polyuria when given intranasally (1 23) No side effects during DDAVP treatment have been reported so far Therefore this preparation should prove a useful alternative to other forms of treatment for diabetes insipidus in children The present study reports the effects of DDAVP in 10 children suffering from diabetes insipidus caused by deficient secretion of antidiuretic hormone

MATERIAL

Ten patients (4 girls and 6 boys) aged 3 5 to 15 years were included in the study In 7 of them the diabetes insipidus was of the organic type and 3 patients were classified as idiopathic diabetes insipidus (Table 1) In 5 children hypofunction of the adrenal and thyroid glands had been diagnosed before the study These patients were adequately substituted with hydrocortisone and L thyroxine In addition 1 patient was treated with human growth hormone and another received cyclophosphamide therapy All had previously been tested with pitressin tannate in oil or LVP and proved to be vasopressin sensitive

Six of the children were treated with LVP intranasally but in no case could a satisfactory control of thirst and polyuria be obtained with this preparation Especially during night the frequent voidings were disturbing for the older children The small children had a very pronounced nocturnal enuresis

In 4 cases the LVP therapy was particularly unsatisfactory Patient M N experienced the pressor effects of this preparation and complained of headache and abdominal discomfort after each administration Tiredness nausea and poor appetite accompanied the use of LVP in patient L N Parents of J B reported that periodically LVP therapy was totally ineffective Attempts were made with pitressin tannate in oil injections but she rejected this preparation The copious nocturnal enuresis of patient H A was particularly troublesome and could not be controlled by LVP

1 33 The number are however too small for statistical work up The figures are not in accord with the somewhat larger material of Carter (3) where with 281 former male patients the risk for the sons was 1 16 and that for the daugthers, 1 39

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Intranasally administered DDAVP in doses of 2.5–10 μg also had a prompt effect with a long duration of action. In three of the investigated patients a comparison was made between DDAVP and LVP (Table 3, Fig 2). As can be seen, the effect of 20 μg of LVP intranasally was very brief (especially in patient PH) compared with the action of 2.5 μg of DDAVP.

2. Effects of increasing intranasal doses of DDAVP

When DDAVP was administered intranasally twice a day in increasing doses, a dose dependent response was obtained in each of the 8 patients, as can be seen in Fig 3. The osmolality of the daily urine output could be kept at about 500 mOsm/kg H_2O by a median dose of DDAVP of 5 μg × 2 (range 1.25–15 μg × 2). These patients were given 5 μg × 2 (range 1.25–10 μg × 2) as a maintenance dose. With this dosage the children drank normal amounts and their diuresis was less than 1.0–1.5 l/day.

So far the children have been treated for 8–19 months. During this time routine blood and urine laboratory data and also serum sodium were within normal limits. Mean weight gain and growth velocity were in the normal range both before and during DDAVP

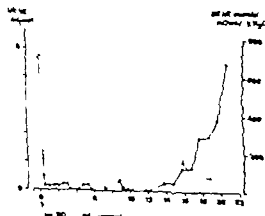


Fig 1. The effects of 1 μg of DDAVP intravenously on urine flow (●—●) and urine osmolality (○ ○) in patient M N

Table 2. *Duration of the effects of 1 μg of DDAVP intravenously*

Patient	Urine flow <1 ml/min (hours)	Urine flow ≈ starting level (hours)	Urine osmolality >300 mOsm/kg H_2O (hours)
P L	7.3	11.2	7.2
P H	5.6	9.0	6.9
M N	16.4	20.2	14.2
H P	9.1	13.0	11.2
H A	7.4	9.7	7.7
Mean	9.2	12.6	9.4

Test performed after second operation of craniopharyngioma

treatment. Night sleep became undisturbed for all the children and their families, and the nocturnal enuresis of the younger children disappeared. In 1 patient (H A) a genuine enuresis remained; this is tolerable for the environment. It is interesting to note that DDAVP even in doses of 40 μg given in the evening did not relieve the enuresis of this patient.

The method of administration has not presented any problems, and there has been no need to change the maintenance doses except during periods of rhinitis, when a slight increase is sometimes necessary. One patient (H P), however, experienced a consistent decrease in the effect of the maintenance dose of DDAVP (Fig 4). Investigations revealed that the craniopharyngioma of this patient had recurred and had to be re-operated. Since operation the need for DDAVP has been constant.

In no patient were the side effects common to LVP and pitressin—such as pallor, headache and abdominal discomfort—observed, nor were there any side reactions from the nasal mucosa.

DISCUSSION

In the present investigation a synthetic analogue of vasopressin, DDAVP, which has a prolonged and specific antidiuretic action, was tested in 10 children. Between 8-arginine vaso-

Table 1 *Relevant data of patients*

Patient	Sex	Age (years)	Diagnosis	Previous antidiuretic treatment	Additional treatment
H P	M	5	Craniopharyngioma operata	LVP	L thyroxin hydrocortisone
H Å	F	5½	Craniopharyngioma operata	LVP PTO (rejected) chlorthalidone + potassium	L thyroxin hydrocortisone
J B	F	7½	Craniopharyngioma operata	LVP PTO (rejected)	L thyroxin hydrocortisone
P L	F	15	Craniopharyngioma operata	LVP chlorthalidone + potassium	L thyroxin, hydrocortisone human growth hormone
M N	M	3½	Idiopathic DI	LVP	
M M	M	4	Idiopathic DI	None	
P K	M	9½	Idiopathic DI	None	
P H	M	3½	Hand-Schuller-Christian s disease?	None	
K L	F	5	Hand-Schuller-Christian s disease	Chlorthalidone + potassium	Cyclophosphamide
L N	M	8½	Medulloblastoma radiotracta	LVP	L thyroxin hydrocortisone

METHODS

Before the administration of DDAVP previous antidiuretic therapy of the children was withdrawn for 2-3 days and during this control period the volume and osmolality of the daily urine output were measured Two series of trials were performed

1 Single intravenous and intranasal doses of DDAVP (5 patients)

On the day of the experiment after the bladder was emptied each patient was given 20 ml of water per kg body weight during 1 hour Urine was collected at 15 min intervals and the volume and osmolality of each sample were determined Two patients were catheterized and 3 voided spontaneously After at least three 15 min periods with a constant diuresis DDAVP was given intravenously in a dose of 1 μg to patients P L P H M N H P and H Å In addition patients P H M N and H Å were given DDAVP intranasally in doses of 2 5-10 μg Patient H Å was tested twice with an interval of 10 months Intravenous and intranasal administrations of DDAVP were performed on separate days The same 3 patients were tested also with LVP 20 μg intranasally

After administration of the preparation urine was collected with intervals of 15-60 minutes and the volume and osmolality of the samples were determined The patients were allowed to drink freely but not less than the amount of urine produced Urine collection was continued until the volume tended to reach the starting level

2 Increasing intranasal doses of DDAVP (10 patients)

In this series of trials all the patients were given five different doses of DDAVP intranasally on separate days in order to determine the smallest effective dose for each patient Each dose was tested for 2 days and the daily water intake urine volume and urine osmolality of the patients were determined DDAVP was administered by means of a graded nasal tube (rhinyle) the doses given being 1 25 2 5 5 10 and in some cases 20 μg twice a day with a 12 hour interval For these trials it was necessary to use two different concentrations of DDAVP 0 1 mg/ml when 10 or 20 μg was given and 0 025 mg/ml when 1 25-5 μg was given Usually the children who were more than 5 years of age could make the insufflations by themselves

RESULTS

1 *Effects of a single dose of DDAVP*

After intravenous administration of 1 μg of DDAVP the urine flow was reduced to less than 1 ml/min and the urine osmolality began to increase within 15–30 min in all 5 patients tested These effects are illustrated in Fig 1 (patient MN) and are summarized in Table 2 The duration of the effect was variable and ranged from 5 6 to 16 4 hours (mean 9 2 hours) if measured as the time when urine flow was less than 1 ml/min There was no definite correlation between on one hand duration of action and on the other body weight or body surface No side effects were observed during these trials

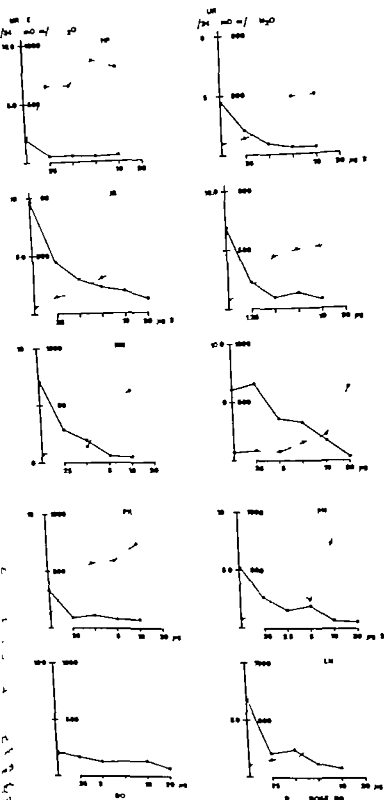


Fig. 3. Effects of increasing doses of DDAVP intranasally on daily urine production (●—●) and urine osmolality (○ ○) in the patients. Each point represents mean of values obtained during two consecutive days of testing.

Table 3 *Duration of the effects of 2 5 μg of DDAVP and 20 μg of LVP*

Patient	Dose (μg)	Urine flow <1 ml/min (hours)	Urine flow = starting level (hours)	Urine osmolality >300 mOsm/kg H_2O (hours)
P H	DDAVP 2 5	7 6	11 8	5 0
M N	DDAVP 2 5	15 6	18 4	15 9
H Å	DDAVP 2 5	15 7	19 0	15 1
H Å	DDAVP 2 5	12 4	14 0	12 7
P H	LVP 20	0	1 5	0
M N	LVP 20	2 0	3 7	0 5
H Å	LVP 20	2 9	4 9	3 0

pressin (AVP) the hormone naturally occurring in man and DDAVP there are two structural differences (Fig 5) In position 1 DDAVP has deaminocystein replacing the hemicysteine in AVP and in position 8,

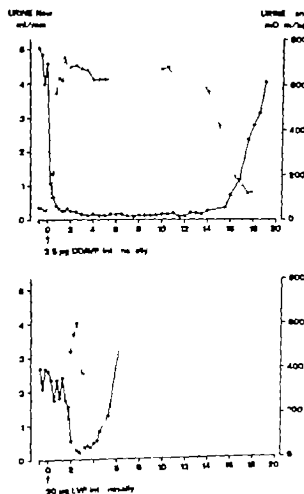


Fig 2 The effects of 2 5 μg of DDAVP intranasally (above) and of 20 μg of LVP intranasally (below) on urine flow (●—●) and urine osmolality (○ ○) in patient H Å

DDAVP has D-arginine instead of the L arginine of the natural hormone Both these changes may contribute to its prolonged action, probably by delaying its enzymatic degradation in the tissues (23) The high antidiuretic activity of DDAVP can to some extent be ascribed to the deamination in position 1 (cys¹) a change which often increases the ratio antidiuretic/vasopressor effect in vasopressin analogues mainly by enhancement of the antidiuretic activity (7) The antidiuretic/vasopressor ratio of AVP is 1 (2) for DDAVP it can be estimated at 2 500–4 500 in the doses used in the present study (23) As the replacement of L forms of amino acids by D-forms in position 8 is usually associated with a reduction of the pressor effects of vasopressin (26 27) this structural change in DDAVP might contribute to its very low pressor activity

The value of DDAVP in the treatment of diabetes insipidus due to lack of vasopressin has previously been documented mainly in adults (1 23) In the present investigation its effects in children with this disease were further elucidated It was found that in all the patients tested intranasal administration of DDAVP 1 25–10 0 μg twice a day was sufficient to normalize drinking and urine production and in no case was injection therapy necessary No relation was found between the dose needed and the body size This suggests that the effect of a given dose of DDAVP

propamide is ineffective and often its effect is only moderate (9 15 20) Clofibrate (Atromide®) and carbamazepine (Tegretol®) have very few side effects but neither substance is sufficiently effective in all cases of the disease (4 5 20 21) Thiazide diuretics have a well known antidiuretic action in both nephrogenic diabetes insipidus and in patients with deficiency of vasopressin but their effects are seldom sufficient to normalize urine production and hypopotassaemia is a frequent side effect (10)

A good control of the polyuria and polydipsia of diabetes insipidus is of particular importance in childhood As shown by Vest et al (24) uncompensated diabetes insipidus may lead to stunting of growth (hypocaloric dwarfism) and hydronephrosis Adequate vasopressin substitution can normalize growth and the changes in the urinary tract can be reversed at least in their early phases (12 24) Pitressin tannate in oil has so far been the only vasopressin preparation available for producing a reliable and long lasting antidiuretic effect which is necessary to prevent this type of dwarfism However the well known side effects of the preparation cause some patients to renounce its permanent use The ease of administration lack of side effects and long duration of action make DDAVP a valuable alternative in the treatment of diabetes insipidus in children Judged from continuous treatment over a period of 8–19 months it is particularly well suited for long term use

SUMMARY

The effects of a new synthetic analogue of vasopressin DDAVP (1 deamino 8 D arginine vasopressin) was investigated in 10 children with diabetes insipidus due to deficient secretion of antidiuretic hormone The lack of pressor activity and the specific and long lasting antidiuretic effect of this preparation was confirmed During an observation period of 8–19 months it was found that intranasal administration of 1 25–10 μg of DDAVP twice daily was sufficient to normalize drinking and urine production in all the patients No side effects were observed It is concluded that DDAVP is a valuable alternative in the treatment of vasopressin sensitive diabetes insipidus in children and that it is well suited for long term use

ACKNOWLEDGEMENT

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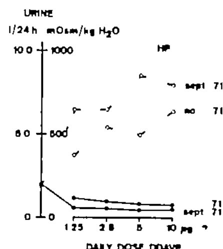


Fig 4 Decreased effect of DDAVP in patient H P when tested in September and November 1971 During this time a return of the craniopharyngioma of the patient was detected Symbols as in Fig 3

depends on several factors One of these seems to be the intranasal resorption which may be variable e g during periods of rhinitis Individual variations in kidney sensitivity to DDAVP and the ability to degrade the peptide might also influence the effect and perhaps explain the wide range duration of the antidiuretic action from 5 6 to 16 3 hours after 1 μg intravenously Finally other factors might be of importance In patient H P the recurrence of his craniopharyngioma was associated with an increase in the need for DDAVP

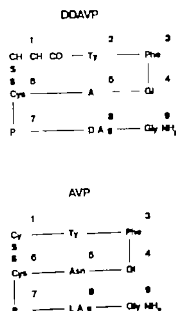


Fig 5 Chemical structures of DDAVP and of AVP

We have not so far observed any side effects during DDAVP treatment Intravenous administration of 1 μg did not produce any pressor reactions, nor did doses of 40 μg given intranasally No headache abdominal discomfort and pallor of the skin complaints common with LVP and pitressin treatment were seen The mode of administration seemed to offer no problems and the diminished resorption of DDAVP during infections of the upper respiratory tract was easily overcome with a slight increase in the dose

Rhinitis and nasal congestion are often encountered with posterior pituitary snuff but to a great extent can be avoided with pure synthetic preparations (3 11) Antibody formation and allergic reactions after administration of synthetic neurohypophyseal hormones seem to be extremely rare (13 14 18) in these respects therefore DDAVP can be expected to be a safe preparation

The high antidiuretic activity of DDAVP may raise the question whether there is a risk of water intoxication at overdosage of the preparation Though not observed by us this complication should be kept in mind With the doses used in the present study the risk is probably very small and this kind of complication might be avoided if the starting dose is low (1 25–2 5 $\mu g \times 2$) at the beginning of the treatment

All the oral antidiuretics at present in use as alternatives to vasopressin therapy in the treatment of diabetes insipidus have one advantage over DDAVP the more simple route of administration None of these drugs however are ideal because of their side effects and unpredictable actions Chlorpropamide, which has gained wide use in the treatment of diabetes insipidus in adults has a tendency to produce hypoglycemia in children (6 8 9 15 16 19 22) Patients with anterior pituitary insufficiency seem to be particularly sensitive to this action (9 25) and the use of chlorpropamide in childhood diabetes insipidus may be questioned Furthermore in some cases of vasopressin sensitive diabetes insipidus chlor

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THE EFFECT OF MODERATE HYPOCAPNIA ON THE CEREBRAL ARTERIO VENOUS DIFFERENCE OF ACETOACETATE D β HYDROXYBUTYRATE AND OXYGEN IN CHILDREN

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In a recent study we described significant cerebral arteriovenous differences of acetoacetate and D β hydroxybutyrate which were proportional to their arterial concentrations in children after a relative short period of fasting (7) Significant cerebral arterio-venous differences were also found for glucose and oxygen while the arterio venous differences of free fatty acids (FFA) glycerol lactate and pyruvate were non significant The present study deals with the influence of variations in cerebral blood flow on the cerebral arterio venous differences of ketone bodies Studies in adult humans and animals have shown that moderate hypocapnia induced by passive hyperventilation decreases cerebral blood flow without affecting the cerebral metabolic rate of oxygen (1 5 10 11) The values for the cerebral arterio-venous difference of oxygen determined in normocapnia and hypocapnia were used to calculate the cerebral flow *equivalents* (CFE) The influence of a reduced cerebral blood flow during hypocapnia on the uptake or production of metabolites were calculated by multiplying their cerebral arterio-venous *differences* by the respective cerebral flow equivalents

MATERIAL AND METHODS

Seven children ages ranging from 7 months to 14 years, were studied during anaesthesia before surgery or X-ray investigation Two of them were also studied within 2 hours after anaesthesia, when awake After pre medication with morphine and atropine (2) and induction with thiopenthone the anaesthetic technique used was nitrous oxide (70) oxygen (30%) relaxant, intubation and controlled ventilation with a volume timecycled ventilator (UR 70 CAMECO Stockholm Sweden) Blood samples were drawn from catheters percutaneously introduced into the radial artery (Venflon 100 O D 1 0 mm Viggo Halsingborg Sweden) and into the superior bulb of the internal jugular vein (Nylon intravenous cannula "Green FG O D 0 63 mm Portex Hythe Kent England) The first sampling period was during normocapnia the second after 15 minutes of hypocapnia Four or five paired blood samples were drawn from each patient during normo- and hypocapnia pH base excess P_{O_2} P_{CO_2} and oxygen content were determined in all samples The samples obtained from 3 patients were analysed for lactate and pyruvate in blood and for glucose acetoacetate D-β hydroxybutyrate glycerol and FFA in plasma Sampling technique and analytical methods were the same as previously described (7)

RESULTS

The 3 patients age range 1–11 years in whom all parameters were analysed had a mean arterial P_{CO_2} ±S E of 41 4±0 8 mmHg during normocapnia and 20 0±0 7 mmHg during hypocapnia The mean arterial concentrations of glucose acetoacetate D-β hydroxybutyrate and glycerol were unaffected by hypocapnia while the mean concentrations of FFA lactate and pyruvate increased significantly (Table 1) Using Student s paired *t* test significant cerebral arterio-venous differences were found for glucose acetoacetate and D β hydroxybutyrate

Table 1 *Arterial concentrations of metabolites during normocapnia and hypocapnia*

Values are expressed as mean ± S E of 13 observations Concentrations are given in mM

	Normocapnia[a]	p[b]	Hypocapnia[c]
Glucose	4.18±0.36	NS	4.10±0.29
Acetoacetate	0.60±0.104	NS	0.59±0.071
D β hydroxybutyrate	1.75±0.26	NS	2.23±0.26
FFA	1.47±0.01	<0.001	1.76±0.07
Glycerol	0.27±0.03	NS	0.34±0.02
Lactate	1.52±0.17	<0.02	2.64±0.41
Pyruvate	0.08±0.01	<0.02	0.12±0.01

[a] P_{aO_2} were above 100 mmHg in all samples giving an oxyhaemoglobin saturation above 95

[b] p indicates statistical differences between mean values

[c] P_{aCO_2} was significantly reduced during hypocapnia ($p<0.001$) and P_{aO_2} were above 100 mmHg in all samples

both during normo and hypocapnia (Table 2) A highly significant ($p<0.001$) negative arterio-venous difference was found for lactate only during hypocapnia The mean arterio venous differences of oxygen glucose and lactate increased significantly during hypocapnia (Table 2)

The relationship between P_{aCO_2} (x) and the calculated cerebral flow equivalent (y CFE = ml perfused blood per ml O_2 consumed) are given in Fig 1 CFE values at different P_{aCO_2} values have been calculated from published data on anaesthetized dogs (10) and awake adult humans (5) during passive hyperventilation These values have been included in Fig 1 The equation of the regression line for the correlation between P_{aCO_2} (x) and CFE (y) was in our material $y=1.8+0.71x$ ($r=0.89$ $n=50$ $p<0.001$) The corresponding equation of the regression line for adult humans was $y=2.15+0.29x$ ($r=0.90$ $n=16$ $p<0.001$) and for adult dogs $y=5.8+0.17x$ ($r=0.72$, $n=37$ $p<0.001$) These regression lines are also included in Fig 1 A fourth regression line $y=-0.6+0.44x$ has been included in Fig 1 It refers to adult humans anaesthetized with the same technique as used in the present study i e 70% nitrous oxide 30% oxygen and relaxant The equation has been calculated from mean values of cerebral blood flow and cerebral metabolic rate of oxygen during normo- and hypocapnia (11)

When the cerebral arterio venous differences of substrates determined were multiplied by the respective cerebral flow equivalents significant reductions of the relative uptakes of acetoacetate and D β hydroxybutyrate were found during hypocapnia while the relative uptake of glucose and the production of lactate were not significantly altered (Table 3) The lactate/pyruvate ratios in arterial and venous blood during normocapia were 20.02±2.74 and 19.06±2.67 (mean ± S E) respectively The corresponding values during hypocapnia were 21.56±1.72 and 20.85±1.66 respectively Using the paired t test no significant change in the lactate/pyruvate ratios between arterial and

Table 2 *Cerebral arterio venous differences of metabolites and oxygen during normocapnia and hypocapnia*

Values are expressed as mean ±S E of 13 observations Concentrations are given in mM

	Normocapnia	p[a]	Hypocapnia	p[a]	p[b]
Glucose	0.29+0.04	<0.001	0.67±0.08	<0.001	<0.001
Acetoacetate	0.08±0.01	<0.001	0.07±0.01	<0.001	NS
D β hydroxybutyrate	0.09±0.02	<0.01	0.10+0.01	<0.001	NS
FFA	0.02±0.00	NS	0.02±0.00	NS	NS
Glycerol	0.01±0.00	NS	0.02±0.01	NS	NS
Lactate	0.03±0.01	NS	0.07±0.01	<0.001	<0.01
Pyruvate	0.01±0.00	NS	0.01±0.00	NS	NS
Oxygen	1.49±0.07	<0.001	4.49±0.09	<0.001	<0.001

[a] p indicates significant cerebral arterio venous differences (paired t test)

[b] p indicates statistical differences between mean values during normo and hypocapnia

venous blood was found during normocapnia as opposite to a significant ($p<0.02$) lower ratio in cerebral venous blood during hypocapnia. During normo- and hypocapnia a significant inverse correlation was found between arterial values of glycerol and FFA ($r=0.72$ and 0.76, $p<0.01$ respectively).

DISCUSSION

It has been repeatedly demonstrated that the cerebral metabolic rate of oxygen is unaffected by moderate hypocapnia in adult humans and animals (1, 5, 10, 11). This implies that the inverted value of the arterio-venous difference of oxygen is directly proportional to cerebral blood flow.

As this is also probably valid in children the present results indicate that the uptake of ketone bodies by the brain is directly proportional to cerebral blood flow. Since the arterio-venous differences of ketone bodies are positively correlated to their arterial concentrations (7) it follows that the uptake is dependent on the amount of ketone bodies supplied to the brain per unit time. The rate of cerebral utilization of ketone bodies is furthermore dependent on the activities of cerebral enzymes involved in ketone body metabolism. Published data also indicate that the arterio-venous difference of ketone bodies (i.e. the sum of acetoacetate and D-β-hydroxybutyrate) at a given arterial concentration is higher in children than in adults (7). The present observation of a higher normocapnic cerebral flow equivalent and reported higher normocapnic cerebral blood flow values in children (4) indicate that at a given arterial concentration of ketone bodies the cerebral uptake would be approximately four times greater in children than in adults.

It is well known that the relationship between P_{aco_2} and cerebral blood flow is not linear. Reivich et al. have treated this relation as if it was linear in order to compare different groups (8). For the same reason we have treated the relation between P_{aco_2} and cerebral flow equivalent as if it was linear. When

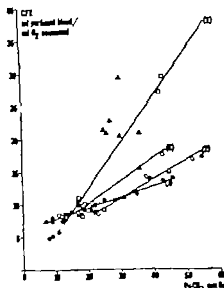


Fig. 1. Cerebral flow equivalent values plotted as a function of P_{aco_2}. (1) Present study: □ Anaesthetized children, △ awake children. (2) ○ Awake adults (5). (3) ● Anaesthetized dogs (10). (4) Adult humans anaesthetized by the same technique as in the present study (70% N_2O, 30% O_2) (11). For equations see text.

comparing the cerebral flow equivalents at P_{aco_2} levels of 40 and 20 mmHg in children and adults anaesthetized by the same technique the mean relative reduction of cerebral flow equivalents were similar: 54 and 52% respectively. This indicates that the reactivity

Table 3. *Calculated changes in uptake and production of metabolites induced by hypocapnia*

Relative values for uptake and production during normocapnia were taken as 100%

	Normocapnia ( )	Hypocapnia (%)	*p*
Uptake			
Glucose	100	80	NS
Acetoacetate	100	27	<0.01
D-β-hydroxybutyrate	100	41	<0.05
Production			
Lactate	100	87	NS

Statistical differences between mean relative values for uptake and production (CFE × arterio-venous differences).

Table 1 Arterial concentrations of metabolites during normocapnia and hypocapnia

Values are expressed as mean ± S.E. of 13 observations. Concentrations are given in mM

	Normo-capnia[a]	p[b]	Hypo-capnia[c]
Glucose	4.18±0.36	NS	4.10±0.29
Acetoacetate	0.60±0.104	NS	0.59±0.071
D-β hydroxy butyrate	1.75±0.76	NS	2.23±0.26
FFA	1.47±0.01	<0.001	1.76±0.07
Glycerol	0.27±0.03	NS	0.34±0.02
Lactate	1.52±0.17	<0.02	2.64±0.41
Pyruvate	0.08±0.01	<0.02	0.12±0.01

[a] $P_{a_{O_2}}$ were above 100 mmHg in all samples giving an oxyhaemoglobin saturation above 95
[b] p indicates statistical differences between mean values
[c] $P_{a_{CO_2}}$ was significantly reduced during hypocapnia ($p<0.001$) and $P_{a_{O_2}}$ were above 100 mmHg in all samples

both during normo- and hypocapnia (Table 2). A highly significant ($p<0.001$) negative arterio-venous difference was found for lactate only during hypocapnia. The mean arterio venous differences of oxygen, glucose and lactate increased significantly during hypocapnia (Table 2).

The relationship between $P_{a_{CO_2}}$ (x) and the calculated cerebral flow equivalent (y, CFE = ml perfused blood per ml O_2 consumed) are given in Fig. 1. CFE values at different $P_{a_{CO_2}}$ values have been calculated from published data on anaesthetized dogs (10) and awake adult humans (5) during passive hyperventilation. These values have been included in Fig. 1. The equation of the regression line for the correlation between $P_{a_{CO_2}}$ (x) and CFE (y) was in our material $y = 1.8+0.71x$ ($r=0.89$, $n=50$, $p<0.001$). The corresponding equation of the regression line for adult humans was $y = 2.15+0.29x$ ($r=0.90$, $n=16$, $p<0.001$) and for adult dogs $y = 5.8+0.17x$ ($r=0.72$, $n=37$, $p<0.001$). These regression lines are also included in Fig. 1. A fourth regression line $y = -0.6+0.44x$ has been included in Fig. 1. It refers to adult humans anaesthetized with the same technique as used in the present study, i.e. 70% nitrous oxide, 30% oxygen and relaxant. The equation has been calculated from mean values of cerebral blood flow and cerebral metabolic rate of oxygen during normo- and hypocapnia (11).

When the cerebral arterio venous differences of substrates determined were multiplied by the respective cerebral flow equivalents significant reductions of the relative uptakes of acetoacetate and D-β hydroxybutyrate were found during hypocapnia, while the relative uptake of glucose and the production of lactate were not significantly altered (Table 3). The lactate/pyruvate ratios in arterial and venous blood during normocapnia were 20.02±2.74 and 19.06±2.67 (mean ± S.E.) respectively. The corresponding values during hypocapnia were 21.56±1.72 and 20.85±1.66 respectively. Using the paired t test no significant change in the lactate/pyruvate ratios between arterial and

Table 2 Cerebral arterio venous differences of metabolites and oxygen during normocapnia and hypocapnia

Values are expressed as mean ±S.E. of 13 observations. Concentrations are given in mM

	Normocapnia	p[a]	Hypocapnia	p[a]	p[b]
Glucose	0.79±0.04	<0.001	0.67±0.08	<0.001	<0.001
Acetoacetate	0.08±0.01	<0.001	0.07±0.01	<0.001	NS
D-β hydroxybutyrate	0.09±0.02	<0.01	0.10±0.01	<0.001	NS
FFA	0.02±0.00	NS	0.02+0.00	NS	NS
Glycerol	0.01±0.00	NS	0.02+0.01	NS	NS
Lactate	0.03±0.01	NS	0.07±0.01	<0.001	<0.01
Pyruvate	0.01±0.00	NS	0.01±0.00	NS	NS
Oxygen	1.49±0.07	<0.001	4.49±0.09	<0.001	<0.001

[a] p indicates significant cerebral arterio-venous differences (paired t test)
[b] p indicates statistical differences between mean values during normo- and hypocapnia

venous blood was found during normocapnia as opposite to a significant ($p < 0.02$) lower ratio in cerebral venous blood during hypocapnia. During normo- and hypocapnia a significant inverse correlation was found between arterial values of glycerol and FFA ($r = 0.72$ and 0.76, $p < 0.01$ respectively).

DISCUSSION

It has been repeatedly demonstrated that the cerebral metabolic rate of oxygen is unaffected by moderate hypocapnia in adult humans and animals (1, 5, 10, 11). This implies that the inverted value of the arterio-venous difference of oxygen is directly proportional to cerebral blood flow.

As this is also probably valid in children the present results indicate that the uptake of ketone bodies by the brain is directly proportional to cerebral blood flow. Since the arterio-venous differences of ketone bodies are positively correlated to their arterial concentrations (7), it follows that the uptake is dependent on the amount of ketone bodies supplied to the brain per unit time. The rate of cerebral utilization of ketone bodies is furthermore dependent on the activities of cerebral enzymes involved in ketone body metabolism. Published data also indicate that the arterio-venous difference of ketone bodies (i.e. the sum of acetoacetate and D-β-hydroxybutyrate) at a given arterial concentration is higher in children than in adults (7). The present observation of a higher normocapnic cerebral flow equivalent and reported higher normocapnic cerebral blood flow values in children (4) indicate that at a given arterial concentration of ketone bodies the cerebral uptake would be approximately four times greater in children than in adults.

It is well known that the relationship between P_{aCO_2} and cerebral blood flow is not linear. Reivich et al. have treated this relation as if it was linear in order to compare different groups (8). For the same reason we have treated the relation between P_{aCO_2} and cerebral flow equivalent as if it was linear. When comparing the cerebral flow equivalents at P_{aCO_2} levels of 40 and 20 mmHg in children and adults anaesthetized by the same technique the mean relative reduction of cerebral flow equivalents were similar: 54 and 52 % respectively. This indicates that the reactivity

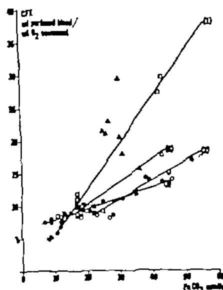


Fig. 1. Cerebral flow equivalent values plotted as a function of P_{aCO_2}. (1) Present study: □ Anaesthetized children, △ awake children. (2) ○ Awake adults (5). (3) ● Anaesthetized dogs (10). (4) Adult humans anaesthetized by the same technique as in the present study (70% N_2O, 30% O_2) (11). For equations see text.

Table 3. *Calculated changes in uptake and production of metabolites induced by hypocapnia*

Relative values for uptake and production during normocapnia were taken as 100 %

	Normocapnia ( )	Hypocapnia (%)	p^a
Uptake			
Glucose	100	80	NS
Acetoacetate	100	27	<0.01
D-β-hydroxybutyrate	100	41	<0.05
Production			
Lactate	100	87	NS

a Statistical differences between mean relative values for uptake and production (CFE × arterio-venous differences).

of the cerebral blood vessels to the vasodilating effect of carbon dioxide is the same in children and adults This finding is somewhat at variance with the observation by Reivich et al who found less reactivity to the vasodilating effect of carbon dioxide in newborn than in adult rhesus monkeys (8) Alkalosis is known to increase the rate of glycolysis in the tissues, including the erythrocytes (9) The H^+ ions liberated from glycolytically generated lactic acid will reduce the pH The increased blood oxygen affinity caused by alkalosis will hereby be counteracted and thus favour the oxygen release to the tissues As has been suggested by Laver lactate production might be regarded as a rapid and easily reversible way of regulating oxygen release (6)

Differences in the lactate/pyruvate ratios between arterial and venous blood are probably a poor estimate of intracellular changes At $P_{a_{CO_2}}$ levels below 20 mmHg a steep increase in calculated intracellular lactate/pyruvate ratios has been demonstrated in animal experiments while the ratio between ATP ADP and AMP remained normal and unchanged (3) These results were interpreted as possibly indicating moderate brain hypoxia at $P_{a_{CO}}$ levels well below 20 mmHg

Further studies are needed to clarify the mechanism behind the observed increase in mean arterial concentrations of FFA which occurred at unchanged mean levels of glycerol and ketone bodies during hypocapnia

SUMMARY

Cerebral arterio-venous differences of acetoacetate D-β hydroxybutyrate glucose glycerol FFA lactate pyruvate and oxygen were determined during normo and hypocapnia in children anaesthetized in connection with surgical operations or X ray procedures Cerebral flow equivalent values were used to calculate the relative changes in uptake or production of substrates during hypocapnia The uptake of ketone bodies was proportional to the cerebral blood flow and to the arterial concentration In comparison with reported values in adults the estimated uptake of ketone bodies at a given arterial concentration was about four times higher in children During hypocapnia but not during normocapnia children had a significant lactate production The vasodilating effect of carbon dioxide on the cerebral blood vessels seems to be the same in children and adults

ACKNOWLEDGEMENTS

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EFFECT OF LIGHT EXPOSURE ON GUT TRANSIT TIME IN JAUNDICED NEWBORNS

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It is well known that during the course of phototherapy stool colour changes from yellow to green The occurrence of frequent loose green stools commencing a few hours after the beginning of treatment has been observed in several different centers (2) It seems reasonable to assume that photo decomposition products play an important role in the pathogenesis of these loose stools

Onishi and co workers have demonstrated that the green stool color is due to the presence of biliverdin (4) They have suggested that biliverdin is increased in the bile by light exposure Furthermore it has also been shown that the photo oxidation of jaundiced skin *in vitro* provokes the formation of bilirubin derivatives having an infrared spectral pattern similar to that of biliverdin (6) *In vivo* the products of the photo oxidated bilirubin of which the structures are not well known are rapidly eliminated in the bile (3 5 7) Ostrow studying the effect of light on the metabolism of ^{14}C-bilirubin in Gunn rats found only small quantities of radioactivity in the urine mainly attributable to dipyrroles whereas the major portion of the radioactivity proved to be present in the bile as unconjugated bilirubin and unidentified bilirubin derivatives (5)

The purpose of the present study is to investigate the effect of light exposure on gut transit time in jaundiced newborns

MATERIALS AND METHODS

The following groups of experiments were performed in the course of the present study

Group I 13 full term healthy newborns betwe[en] 3 and 5 days of age were subjected to the Carmin[e] test in order to determine normal gut transit time

Group II 12 full term jaundiced newborns havi[ng] the same age as those in group I with a mean un[-] conjugated bilirubin level of 18 mg/100 ml and with[-] out clinical signs of infection were administered th[e] Carmine test both before and during the cours[e] o[f] phototherapy which was started 24 hours previou[sly] These newborns (naked except for a blindfold) wer[e] continuously exposed to light in a phototherapy un[it] supplied with 12 20 W cool white fluorescent tube[s] attached to a wood and aluminum cradle The inten[-] sity of light at the level of the infant was approx[-] imately 500 foot candles

Group III 10 full term healthy newborns tha[t] underwent 48 hours of phototherapy The Carmin[e] test was performed 24 hours after the beginning o[f] treatment

Carmine test was used to study intestinal transi[t] time It is based on the appearance of the first re[d] stool after administration of food to which 50 mg o[f] Carmine Red has been added

RESULTS

Group I the results obtained studying in[-] testinal transit time by means of the Carmine test indicate that the speed of complete in[-] testinal transit in normal newborns in our experience is 13 14 ± 4 43 hours (Fig 1)

Group II intestinal transit time before pho[-] totherapy was normal in all 12 jaundiced new[-] borns However intestinal transit time was accelerated after phototherapy as compared with normal newborns (Fig 1)

Group III phototherapy on healthy new[-] borns had no effect on intestinal transit time (Fig 1)

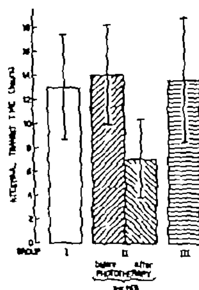


Fig. 1. The intestinal transit time determined with Carmine Red test, in 13 healthy full term newborns (group I), in 12 jaundiced newborns before and after phototherapy (group II), in 10 healthy newborns exposed to phototherapy (group III).

DISCUSSION

Our findings indicate that therapeutic exposure of jaundiced newborns to light provokes an increased rate of intestinal transit and the appearance of loose green stools as compared with normal and jaundiced newborns not exposed to the therapy. These effects were not observerd in non jaundiced newborns. Our data disagree with those of Washington and co-workers who were unable to find a significantly increased incidence of diarrhoea in their investigation (8). Blackburn et al. in a study on the combined effect of phototherapy and phenobarbital on serum bilirubin levels of premature infants reported only the average number of stools ranging from 1.7 to 8.5 omitting the consistency (1). The variation is most likely due to the different indication for light exposure. When phototherapy is applied to prevent hyperbilirubinemia in newborns with an increased risk of developing significant hyperbilirubinemia (low birth weight infants) the appearance of loose stools is not as frequent as in very jaundiced neonates. These findings support the hypothesis that the products of photo-oxidated bilirubin play an important role in the etiology of this diarrhoea. Previous studies moreover indicate that these products are rapidly eliminated in the bile (3). The exact structure of these products is unknown but the photo-oxidation of human jaundiced skin *in vitro* provokes the formation of bilirubin derivatives which have an infrared spectral pattern similar to that of biliverdin (6).

In our experience the occurrence of frequent loose green stools during phototherapy is not a serious contra-indication to light therapy. It should in fact be considered whether the accelerated intestinal transit time is useful through preventing the reabsorption of bilirubin in the intestine in this way inhibiting the entero-hepatic cycle of bilirubin.

The infusion of photo-oxidated bilirubin into humans was not employed for two reasons: first because of the possible neurotoxic effects of these products and secondly because it has been well demonstrated that the water soluble derivatives of bilirubin excreted in the bile of Gunn rats after light exposure are different from the derivatives formed during the photodecomposition of bilirubin *in vitro*.

It seems reasonable to conclude that the diarrhoea which occurs in infants during the course of photo-therapy is a result of the biliary elimination of photo-decomposition derivatives of bilirubin.

SUMMARY

In order to elucidate the mechanism by which phototherapy induces loose stools in newborns studies were performed on the speed of gut transit by performing the Carmine Red test on normal newborn, on jaundiced newborns before and after phototherapy.

A statistically accelerated intestinal transit was observed in jaundiced newborns treated with phototherapy.

The increased rate of intestinal transit produced by phototherapy is probably due to the

action of the photo decomposition derivatives of bilirubin which are excreted during photo therapy

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FIBRINOLYTIC ACTIVITY IN LUNG TISSUE FROM NEONATES WITH HYALINE MEMBRANE DISEASE

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The etiology of the hyaline membrane disease (HMD) remains unknown Many pathogenetic factors have been suggested (23) including a defective fibrinolytic system (1 2 18 19 20 21) leading to permanent deposition in the lungs of hyaline membranes containing fibrin

The fibrinolytic activity of lung tissues from neonates has been measured in lung homogenates (14 21) as well as in saline or potassium thiocyanate extracts of the lungs (2 15 20) on fibrin plates according to Astrup & Mullertz (4) A valuable method for studying fibrinolytic activity in tissues was introduced by Todd in 1959 (28) With this method (fibrinolysis autography technique) it is possible to locate plasminogen activator activity in tissue The method has been further developed by Pandolfi et al (24) and now permits semi quantitative determination of the tissue fibrinolytic activity

In view of the controversial results obtained with the above mentioned extraction methods we thought it worth while to study the fibrinolytic activity in the lungs of newborns with the histochemical technique As far as we know in only one newborn with HMD has the fibrinolytic activity in lung tissue been studied with this technique (25)

This paper reports the findings in 29 newborns with HMD atelectasis and pulmonary haemorrhage

CLINICAL MATERIAL

28 liveborn infants cared for at the Department of Paediatrics and one stillborn infant from the Department of Obstetrics were studied Clinical and pathological data are given in Table 1 The clinical criteria used for idiopathic respiratory distress syndrome (IRDS) were those published by Hutchison et al (13) Chest X ray however was negative in one case and not performed in 5 All the liveborn infants had undergone routine neonatal care including incubator oxygen treatment and intravascular fluid therapy Respirator treatment had never been used One infant (no 27) had been subjected to general anaesthesia for operation because of an abdominal malformation

METHODS

After death the corpses were kept in the wards for up to 2 hours according to regulations and were then transferred to the Department of Pathology Some necropsies were performed immediately otherwise the bodies were kept at +4 C until necropsy The interval between death and necropsy was 1 to 68 hours with a median of 9 hours At the beginning of necropsy pieces about 1 cm across were removed from the lower lobes of both lungs for assessment of the fibrinolytic activity They were fixed immediately by freezing in expanding carbon dioxide and kept at −70 C in plastic bags until sectioned The rest of the lungs and specimens from other organs were f xed in neutralized 10% formaldehyde solution Histological sections were cut from all lung lobes and stained with haematoxylin-eosin (HE) All se tions were studied repeatedly by one of us (G O) without knowledge of the clinical history of the respective cases and the findings were grouped according to special scheme The sections did not vary in appearance with the lobes from which they had been obtained *Normal lungs* were diffusely

Table 1 *Survey of the material and results*

The fibrinolytic activity is expressed in arbitrary units (A U) see text
For explanation of grading of hyaline membranes (HM) see text
IRDS Idiopathic respiratory distress syndrome PH Pulmonary haemorrhage A Alveolar I Interstitial AI Combination of both

Group	Case no	Sex	Birth weight	Week of gestation	Diagnosis	Age (hours)	Necropsy findings HM	PH	Interval death/necropsy (hours)	Fibr activ (A U)
Normal	1	♀	3 000	37	Myelomeningocele	122	—	—	2	6½
	2	♀	3 390	42	Anencephalia	9	—	—	10	7½
Hyaline membranes	3	♂	680	25	Postnatal asphyxia Twin no 2	6	+ + +	—	2	4½
	4	♀	710	25–26	Postnatal asphyxia	7	+ +	—	10	3
	5	o	1 150	26–27	IRDS no X ray	15	+ +	AI	5	½
	6	♂	1 000	27	Postnatal asphyxia	8	+ +	AI	2	9
	7	♀	1 200	28	IRDS no X ray Twin no 1	50	+ +	A	19	0
	8	♀	1 560	30	IRDS (X ray pos)	41	+ + +	—	3	[illegible]
	9	o	1 500	31	Postnatal asphyxia Twin no 2	91	+ +	—	11	1
	10	o	1 200	31	IRDS no X ray	9	+ + +	—	7	4½
	11	o	1 570	32	IRDS (X ray pos) Twin no 2	26	+ + +	I	4	3
	12	♂	2 180	32	Postnatal asphyxia	5	+	—	9	0
	13	♀	2 190	32	IRDS (X ray pos) Twin no 2	18	+ + +	—	1	0
	14	♀	1 840	32	IRDS no X ray	11	+	—	13	0
	15	♂	1 690	33	IRDS (X ray neg)	13	+ +	I	6	0
	16	♂	2 150	34	IRDS no X ray	6	+ +	I	8	4
	17	♀	2 200	34	IRDS (X ray pos)	50	+ + +	—	43	3½
Atelectasis without hyaline membrane	18	♀	640	24	Immaturity	62	—	—	18	8
	19	♀	600	25	Postnatal asphyxia	32	—	—	9	4
	20	♂	650	25	Postnatal asphyxia Twin no 2	9	—	—	58	½
	21		830	26	Postnatal asphyxia	67	—	—	5	5
	22	♂	1 170	28	Hyperbilirubinaemia Kernicterus	120	—	—	36	3
	23	♂	1 110	29	Postnatal asphyxia	8	—	A	3	4½
	24	♂	1 240	30	Postnatal asphyxia	10	—	—	45	3½
	25	♀	1 300	31	Postnatal asphyxia	1	—	A	14	8½
	26	♂	2 200	34	Septichaemia + meningitis	28	—	AI	8	0
	27	♀	2 510	36	Gastroschisis + congen toxoplasmosis	170	—	—	6	2
	28	♀	1 280	38	Foetus mortuus + Acrania	—	—	I	68	4½
Massive pulmonary haemorrh	29	o	3 620	40	Apnoea convulsions	4	—	AI	14	9

well aerated with open alveolar ducts and alveoli with thin mostly wavy walls *Atelectasis* was said to be present when only some groups of alveolar ducts and alveoli were open mostly with straight margins and thick walls while in other parts of the lungs the alveoli were completely collapsed Cases with hyaline membranes were not included in this group *Hyaline membranes* were graded according to type and occurrence scattered acidophile deposits in some of the ducts and alveoli (+) more widespread but thin and not coalescent membranes (+ +) and massive thick membranes in alveolar ducts and groups

Fig 1 Normal lung (Case 1) Section processed according to the fibrin slide technique Polymorphous areas of fibrinolysis (pale areas) in areated lung tissue and in connective tissue surrounding a major bronchus (upper right) Incubation time 30 min ×16 *Inset* Areated lung with open alveolar ducts and alveoli with wavy contour thin septa HE ×120

Fig 2 Hyaline membrane lung (Case 6) Wide areas of fibrinolysis Incubation time 30 min ×16 *Inset* Lung with partly expanded alveolar ducts In some of these hyaline deposits or irregular membranes HE ×300

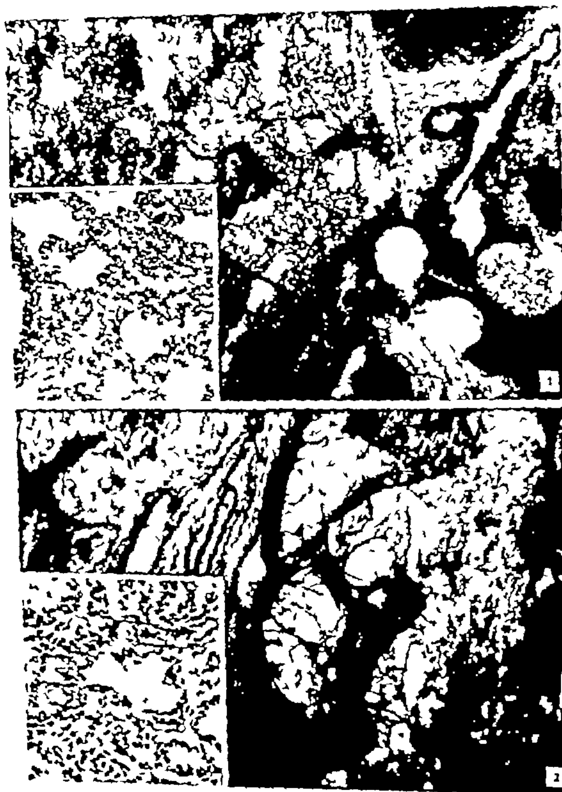


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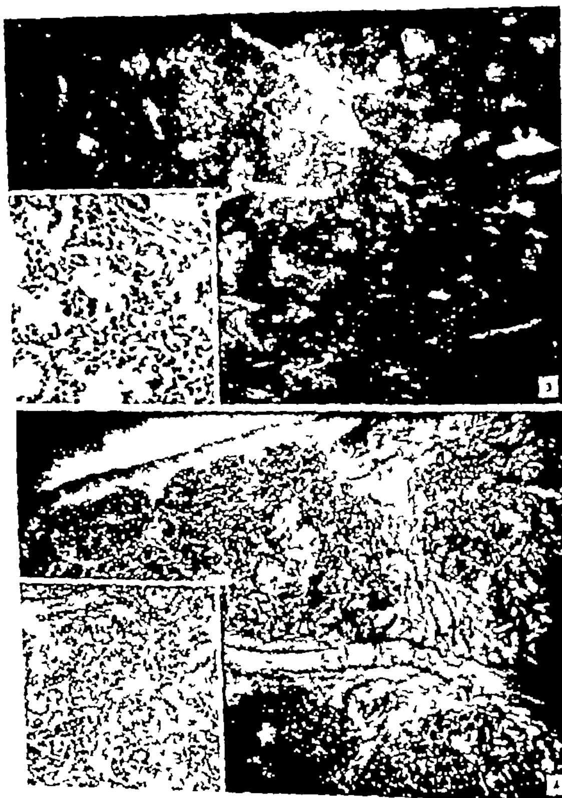
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	9	♂	1 500	31	Postnatal asphyxia Twin no 2	91	+ +	—	11	1
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	11	♂	1 570	32	IRDS (X ray pos) Twin no 2	26	+ + +	I	4	3
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	15	♂	1 650	33	IRDS (X ray neg)	13	+ +	I	6	0
	16	♂	2 150	34	IRDS no X ray	6	+ +	I	8	4
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	21	♀	830	26	Postnatal asphyxia	67	—	—	5	5
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well aerated with open alveolar ducts and alveoli with thin mostly wavy walls *Atelectasis* was said to be present when only some groups of alveolar ducts and alveoli were open mostly with straight margins and thick walls while in other parts of the lungs the alveoli were completely collapsed Cases with hyaline membranes were not included in this group *Hyaline membranes* were graded according to type and occurrence scattered acidophile deposits in some of the ducts and alveoli (+) more widespread but thin and not coalescent membranes (+ +) and massive thick membranes in alveolar ducts and groups

Fig 1 Normal lung (Case 1) Section processed according to the fibrin slide technique Polymorphous areas of fibrinolysis (pale areas) in areated lung tissue and in connective tissue surrounding a major bronchus (upper right) Incubation time 30 min × 16 *Inset* Areated lung with open alveolar ducts and alveoli with wavy contour thin septa HE × 120

Fig 2 Hyaline membrane lung (Case 6) Wide areas of fibrinolysis Incubation time 30 min × 16 *Inset* Lung with partly expanded alveolar ducts In some of these hyaline deposits or irregular membranes HE 300


3
4

of alveoli in all parts of the lungs (+ + +) The findings are given in Table 1 *Pulmonary hemorrhage* was classified as alveolar (A) or interstitial (I) or as a combination of both (AI) Massive pulmonary haemorrhage present in case 29 was defined as diffuse haemorrhages in septa and alveoli in more than one lung lobe The findings are given in Table I

Fibrin slide method

The tissue specimens were examined with a modification (24) of Todd's fibrin slide method (28) which permits localization and assay of fibrinolytic activity in tissue In this method frozen tissue sections (6-8 μm) are incubated in contact with a fibrin film rich in plasminogen During incubation the plasminogen activator in the active cells of the section converts the plasminogen of the fibrin film into plasmin with focal fibrinolysis as a result Subsequent staining reveals the areas of fibrinolysis as round defects in the fibrin film A series of four slides was prepared for each specimen The slides of each series were incubated at 37 C for increasing periods of time i e 15 30 45 60 minutes respectively and afterwards fixed in formalin and stained with Harris haematoxylin Three fairly distinct degrees of fibrin digestion were recognised namely grade I microscopical punctate areas of lysis in most of the sections grade II gross lytic areas of irregular outline and sometimes confluent grade III dissolution of most or all the fibrin in contact with the sections A grade I slide was allotted 1 point a grade II slide 2 points and a grade III 3 points The sum of points (arbitrary units AU) scored by the set of four slides was taken as a measure of the fibrinolytic activity of the sample All specimens were evaluated by one of us (M P) who was unaware of the clinical histories of the cases studied

RESULTS

The results concerning the strength of fibrinolytic activity found in the lungs are given in Table 1

Normal lungs (cases 1 and 2) These cases showed largely equal activity 6 ½ and 7 ½ A U respectively Case 1 is illustrated in Fig 1

Hyaline membrane lungs (15 cases nos 3–17) 5 of these cases showed no activity at all 9 cases ranged between ½ and 4 ½ A U In one infant (no 6 Fig 2) the activity was as high as 9 A U The infant had had severe postnatal asphyxia but no typical signs of IRDS But histological examination demonstrated hyaline membranes Alveolar and/or interstitial haemorrhage were found in 6 cases Generally speaking no relation was found between grad of hyaline membranes and fibrinolytic activity

Atelectatic lungs without hyaline membrane (11 cases nos 18–28) One of these showe no activity In 9 of the cases the activit ranged from ½ to 8 ½ A U This latter case presented in Fig 3 The lung tissue from th stillborn infant had an activity of 4 ½ A U (Fig 4)

Massive pulmonary haemorrhage (case 29 In this case the fibrinolytic activity was hi (9 A U) Histological examination reveale widespread alveolar and interstitial haemo rhages (Fig 5)

The fibrinolytic activity in the atelectat lungs of the liveborn infants (median val 3 75 A U) was higher than in the hyalin membrane lungs (median 2 A U) This di ference studied by the Wilcoxon rank su test was, however, not significant The media activity in these two groups was lower th that in the normal lungs

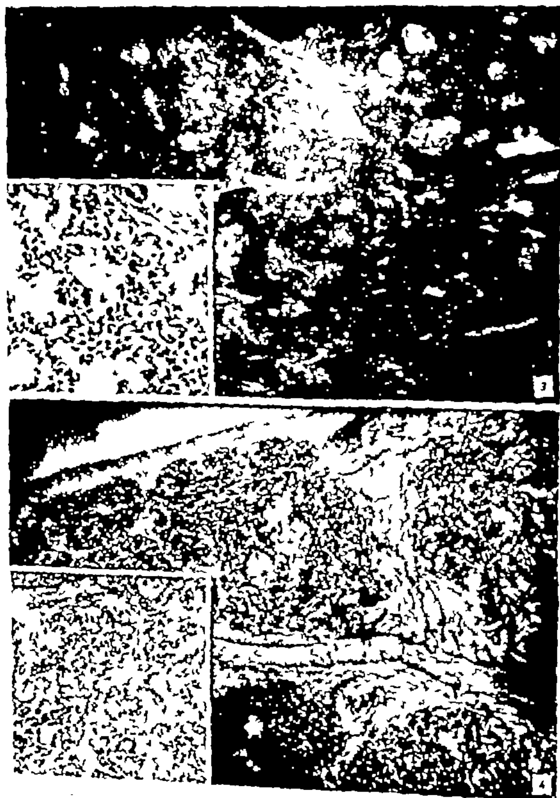
No relation was found between the fibrin lytic activity in the material as a whole an gestational age complications during pre nancy/delivery or Apgar score Neither w any relation found between fibrinolytic acti ity and the interval between death and ne ropsy

DISCUSSION

It has been postulated that a deficiency of f brinolytic activity is one of the pathogenet factors in the development of the hyalin membrane disease (HMD) Ambrus et al (1

Fig 3 Atelectatic lung without hyaline membran (Case 25) Discrete areas of fibrinolysis after the incubation time of 15 min × 10 *Inset* Atelectat lung with collapsed alveolar ducts and alveoli N true hyaline membranes Preserved low epitheliu capillaries containing red blood corpuscles HE × 30

Fig 4 Lung from stillborn infant (Case 28) Roun areas of fibrinolysis in active sites of the lung Th size of the lytic areas is moderate with respect to th long incubation time (60 min) × 6 5 *Inset* Collapse lung with hardly discernible alveolar ducts Blood filled capillaries partly desquamated epithelium in thin bronchi HE × 120


3
4

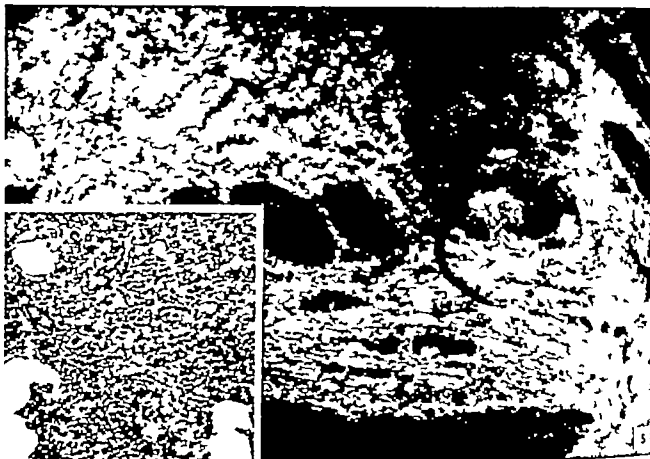


Fig. 5 Massive pulmonary haemorrhage (Case 29) Note the localisation of the activity to small blood vessels Fairly large lytic areas with respect to the short incubation time (15 min) 16 *Inset* Diffuse bleeding with red blood corpuscles in bronchi and alveolar ducts Some open alveoli with rounded margins Red blood corpuscles in oedematous lobular septa HE × 120

studied the fibrinolytic system in blood and in lung tissue specimens of such infants These authors founded their hypothesis on the finding of only very little or even no plasminogen in serum from premature infants a finding which might cause defective fibrinolysis But they could not demonstrate any difference in tissue fibrinolytic activity between normal and hyaline membrane lungs They also demonstrated (3) pulmonary plasminogen activator activity in human foetuses in the fourth month and an increase before term which was interpreted as a physiologic protective mechanism against pulmonary fibrin deposits

Lieberman (18 19 20 21) postulated a deficiency of intrapulmonary fibrinolysis in hyaline membrane lungs and demonstrated a lack of saline soluble plasminogen activator to be the most consistent finding in lungs from newborns with hyaline membranes His results could not be reproduced by other investigators (14 15)

The above mentioned authors used the extraction technique The histochemical method being far less traumatizing for the cells can presumably measure that part of the tissue activator which is most readily available for interaction and therefore of higher physiological value With this method we found no significant difference between a group of hyaline membrane lungs and a group of atelectatic lungs without hyaline membranes Our distinction between hyaline membrane lungs and atelectatic lungs is purely descriptive and does not imply any pathogenetic differences The changes as well as those of massive pulmo-

nary haemorrhage may be variations of a common basic defect in the lungs (abnormal surface forces) as suggested by Rowe & Avery (26) It is true that the activity of the hyaline membrane lungs tended to be lower than that in the atelectatic lungs but in both groups the range of variation was wide One infant (no 6) without typical signs of IRDS had a high activity together with a firm pathoanatomical diagnosis of HMD This finding argues against a defective tissue fibrinolytic activity as an indispensable common factor in the pathogenesis of HMD Our findings are in agreement with those of Roberts (25) He reported an infant with HMD studied with Todd s method where fibrinolytic activity was clearly present in the lungs

The localization of fibrinolytic activity did not significantly differ from that previously observed in the lungs of human foetuses (29) Areas of fibrinolysis were seen scattered over the parenchyma but it was often impossible to recognize the site of origin of the fibrinolytic activity neither was any difference in the pattern of the lytic areas found in the normal and diseased lungs

Of special interest was the high activity in the lungs of the infant with massive pulmonary haemorrhage The etiology of this condition is still completely unknown (27) and we are reluctant to draw any conclusions from this single observation A high fibrinolytic activity may have impaired the local haemostasis and thereby contributed to the haemorrhage

Today it is generally accepted that the hyaline membranes are the result of an intrapulmonary process and at least partly formed by transudation from the damaged alveolar capillaries (10 16 17 27) It is also evident that the membranes are only a secondary phenomenon to some unknown basic process and not the cause of the disease (10 17 22) However it is still a matter of dispute to what extent they contain fibrin According to Gitlin & Craig (12) the membranes consist mainly of fibrin but also fat and squamous cells have been demonstrated Electron microscopical studies by van Breemen et al (8) favoured the opinion of fibrin as a fundamental part of the membranes Schaffer & Avery (27) point out that fibrin is a principal component of the membranes and Bleyl (7) states that they are fibrin rich According to Lauweryns (17) on the other hand fibrin is only exceptionally observed in the membranes which is in agreement with some earlier investigations (5 9) and with histochemical studies by Berezin (6)

The significance of fibrinolysis in the pathogenesis of HMD is thus controversial Lieberman (20) feels that a deficiency of pulmonary plasminogen activator activity though not a primary cause could play a role in the retention of intra pulmonary fibrin deposits and the formation of hyaline membranes We did not study the composition of the membranes Whether fibrin is present in the membranes or not we did not find consistently lower fibrinolytic activity of lung tissue from infants with HMD than from those with atelectasis without hyaline membranes

In conclusion our findings with the histochemical method of Todd question the postulated lack of plasminogen activator in the lung of infants with HMD The results if anything argue against an alteration of the intrapulmonary fibrinolytic system being of any major importance in the formation of hyaline membranes Our findings are also compatible with results obtained in recent investigations of blood in infants with IRDS (11) in which no primary deficiency of the fibrinolytic system in blood could be demonstrated

SUMMARY

The fibrinolytic activity of lung tissue was studied with Todd s histochemical method in a material of 29 newborn infants containing normal lungs hyaline membrane disease atelectasis and massive pulmonary haemorrhage No significant difference was found between a group of hyaline membrane lungs

and a group of atelectatic lungs without hya line membranes In one case with massive pulmonary haemorrhage the activity was high

Our findings make it questionable whether a lack of plasminogen activator in the lung of infants with hyaline membrane disease is a consistent finding and argue against an alteration of the intrapulmonary fibrinolytic system being of any major importance in the formation of the membranes

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5-HYDROXYINDOLEACETIC ACID IN CHILDREN WITH DOWN'S SYNDROME

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Children with Down s syndrome seem to have a defect in their 5 hydroxytryptamine (5 HT) metabolism as indicated by a low level of 5 HT in the blood (5 12 13)

5-HT is synthesized by decarboxylation from 5 hydroxytryptophan (5 HTP) this amino acid in its turn being hydroxylated from tryptophan The active 5 HT which is known as an important neuro transmitter in the central nervous system (CNS) is finally converted to 5-hydroxyindoleacetic acid (5-HIAA) by mono amine oxidase (MAO) This enzyme is bound to mitochondrial structures of the monoaminergic nerves Inhibitors of the enzyme will thus increase the levels of 5 HT both in the peripheral nerves and in the CNS

Heaton Ward (11) reported an improvement of the motor activity and the mental capacity of mongoloid infants after administration of nialamide a MAO inhibitor In 1967 Bazelon et al (6) treated fourteen mongoloid infants with 5 HTP orally The major result of this treatment was an increase in muscle tone

These therapeutic trials were based on the assumption that the defective 5-HT metabolism was shared by the CNS Prompted by the results of earlier investigations indicating that the concentration of 5 HIAA in the cerebro spinal fluid (CSF) is closely related to that in the brain (1 2, 10) we started an investigation of 5 HIAA in CSF in children with Down s syndrome

MATERIAL AND METHODS

Thirty three children with Down s syndrome wer subjected to lumbar puncture and 5 HIAA in CS was determined according to Ashcroft & Sharma (4) The children were between 2 days and 3 5 year old The syndrome was associated with trisomy-(in thirty two cases and with a translocation in one

RESULTS

In Fig 1 the 5-HIAA concentrations in CS from the mongoloid children are plotted i relation to age The values are compared wit the mean level ±2 S D of 102 non hydro cephalic infants (3)

During the first half year of life the 5 HIA concentrations of the mongoloid infants ar in the same range as, or even slightly highe than those of the control group The lev seems to decrease more rapidly than in th controls From the age of 40 weeks and o the 5 HIAA values of the mongoloid childre are significantly lower than the control valu ($p < 0\ 01$)

DISCUSSION

In a previous study on 5 HIAA in lumba CSF (3) where 191 children with and withou hydrocephalus were investigated it could b demonstrated that the 5 HIAA levels in th control non hydrocephalic group were succes sively decreasing and approximating the level of the adults at the age of 40 weeks The cor

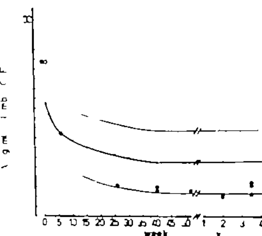


Fig 1 Concentration of 5 HIAA in the CSF related to age in Down s syndrome O Trisomy G ♀ Translocation Solid and broken lines represent mean level and ± S D respectively of 102 controls (Andersson & Roos 1969)

responding values for children with Down s syndrome were equal to and even higher than the control values during the first 25 weeks of life However after about 40 weeks the 5 HIAA levels from the children with Down s syndrome were significantly lower than the control levels

There are at least two possible explanations for the decrease with age from about "normal to subnormal 5 HIAA levels in CSF of mongoloid children

1 The rate of synthesis of 5 HT is normal in the newborn mongoloid child but there is a defective storage of the amine in the granules of the 5 HT neurons In fact defective binding of 5 HT by platelets in Down s syndrome has recently been demonstrated (7) Such a defective binding within the granules of the 5 HT neurons could be compared with the situation after reserpine treatment synthesis is intact but the amine is deaminated and will therefore never be released by the physiological impulse flow The synthesis of 5 HT might be intact but the function defect because of the lack of transmitter released to receptor sites This in turn could lead to an increased synthesis of the amine due to a compensatory feed back mechanism (8) It is possible that this overfunction of the 5 HT synthesizing neurons after some months finally results in an exhaustion showing up as low 5 HIAA levels in CSF at the end of the first year

2 The synthesis of 5 HT (and subsequently 5 HIAA) is impaired in Down s syndrome already at birth but the inefficient transport of 5 HIAA out from the CSF known from other studies in infants (3) keeps the CSF 5 HIAA levels high during the first half year of life The low rate of 5 HIAA production becomes unmasked as low 5 HIAA levels in CSF when the outtransport mechanism gradually turns more efficient Studies are in progress to evaluate this hypothesis using probenecid as a tool

Dubowitz & Rogers (9) were not able to find any difference in CSF 5 HIAA levels between mongoloid children and a control group However this group included children of different ages and in some cases with severe somatic diseases

SUMMARY

The present study has demonstrated the existence of a defect in the metabolism of serotonin in CNS in Down s syndrome The nature of this defect is sofar unknown

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EXCRETION OF BILE ACIDS IN ERYTHROBLASTOSIS FOETALIS

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In intrahepatic cholestasis of infancy ("neonatal hepatitis syndrome") the bile acid excretion to the intestine is greatly diminished or may cease altogether Infants with this syndrome given intramuscularly cholic acid 24-^{14}C excrete the isotope chiefly in the urine (5 6) the bile acid excretion being practically identical with that in extrahepatic biliary atresia (5) This raises the question whether a disturbance of the bile acid excretion is a typical feature of intrahepatic cholestasis of infancy or only of hyperbilirubinaemia in infancy This paper reports the results of studies carried out to investigate this question Infants with erythroblastosis (EB) due to Rh isoimmunization include (i) infants whose serum conjugated bilirubin is increased i e cases of EB associated with cholestasis and (ii) infants in which it is not raised i e cases not associated with cholestasis These two groups were compared with special reference to the isotope excretion the nature of the labelled bile acids present in the urine and the urinary excretion of cholic and chenodeoxycholic acids This enabled further studies of the relationship between increased serum conjugated bilirubin and a disturbance of bile acid metabolism In order to elucidate the part played of EB in the hepatic excretion of bile acids infants with EB not associated with cholestasis were compared with a control group including both normal newborn infants with physiological jaundice and newborn infants with marked hyperbilirubinaemia

CASE MATERIAL

Ten infants with EB due to Rh isoimmunization were studied for 4 days after intramuscular injection of cholic acid 24 C Seven infants had elevated serum conjugated bilirubin indicating that jaundice was complicated by cholestasis Clinical and laboratory findings in the patients and the maternal serum titres of Rh antibodies are given in Table 1 The serum bilirubin concentrations the time of performance of exchange transfusions and of the isotope studies are shown in Fig 1 In all patients the serum transaminase activity (GOT and GPT) leucine-aminopeptidase and gammaglutamyltranspeptidase were within the range considered normal for the neonatal period (3 4 7)

Seven healthy newborn full term male infants were used as controls Their birth weight and birth length were normal Isotope studies were not performed in these infants Five infants were studied from birth until the 4th or the 6th day and the other two from the 1st to the 5th or the 6th day of life Two infants developed marked hyperbilirubinaemia exceeding the values given for physiological jaundice (?) Clinical data regarding the normal infants are given in Table 3 Fig 2 shows the serum bilirubin concentrations None of the infants had elevated serum conjugated bilirubin

METHODS

Cholic acid 4-^{14}C (New England Nuclear Corp Boston Mass) 1 7 5 μCi (0 03–0 05 mg) was given intramuscularly to the infants with EB Urine and faeces were collected daily for 4 days after the injection All patients wore urinary bags and paper diapers

Table 1 *Clinical data in the 10 infants with erythroblastosis*

Patient	Sex	Gestational age (weeks)	Birth weight (g)	Maternal antibody titers of anti Rh	Hemoglobin in cord blood (g/100 ml)	Remarks
L	♀	37	2 460	Albumin 1/2 Papain 1/2048	4 6	Respirator 2nd–3rd day of life because of pulmonary haemorrhage Transfusion of fresh blood
F	♂	38	3 210	Albumin 1/32 Papain 1/4096	6 6	Cesarean section
B	♂	38	3 490	Albumin 1/64 Papain 1/512	5 7	
Å	♂	35	3 260	Albumin 1/8 Papain 1/128	7 0	Cesarean section Hydrops Ampicillin treatment from 4th to 18th day of life
E	♂	39	3 540	Albumin 1/4 Papain 1/256	6 3	Ampicillin treatment from 3rd to 14th day of life
P	♂	36	3 060	Albumin 0 Papain 1/32	7 6	
N	♂	36	2 440	Albumin 1/16 Papain 1/4096	5 8	
F B	♂	36	2 650	Albumin 1/2 Papain 1/256	7 9	
F L	♀	38	2 910	Albumin 1/1 Papain 1/16	n d [a]	
F Å	♀	36	2 200	Albumin 1/8 Papain 1/512	8 1	Cesarean section

[a] Not determined

during the sampling period If the stools were loose the isotope was extracted from the diapers and faeces together As some urine may be passed into the diapers the latter were divided into two groups one including diapers which contained only urine and the other including the diapers containing both urine and faeces Urine faeces and diapers were extracted as previously described and the isotope determined by liquid scintillation technique (5) Ethyl acetate and butanol extracts of urine were analyzed for free and conjugated bile acids by thin layer chromatography (TLC) Radioactive spots were located by autoradiography (5) Urine 30 ml was passed through an Amberlite XAD 2 column and the bile acids eluted with methanol (5) The residue was subjected to solvolysis alkaline hydrolysis and methylation and the final extract was analysed for unconjugated bile acids by TLC Cholic and chenodeoxycholic acids were determined by gas liquid chromatography (GLC) (5)

RESULTS

Infants with Erythroblastosis Foetalis

Isotope excretion

Table 2 gives the recoveries of the administered cholic acid 24 ^{14}C in the urine faeces and in diapers containing only urine and in diapers containing both urine and faeces Only in 4 infants was it possible to separate urine and faeces almost completely In these cases less than 5% of the administered isotope was recovered in the diapers containing both urine and faeces

In infants with EB 33–96% of the isotope was excreted during the 4 days following the injection of cholic acid 24 ^{14}C In the cases of EB associated with cholestasis most of the isotope was recovered in the urine The faeces contained 1–31 of the administered isotope In two cases of EB not complicated by cholestasis similar excretion patterns were observed but patient F A (Table 2) excreted a greater amount of the isotope in the faeces than in the urine The cumulative excretion of isotope was essentially the same irrespective of whether EB was associated with cholestasis (Fig 3 A) or not (Fig 3 B)

Nature of urinary bile acids

TLC analysis showed that small amounts of labelled cholic acid were often present in the

first 24 hour sample of urine No unconjugated labelled cholic acid was detected in samples of urine collected during the second to fourth day after the injection of cholic acid 24-14C

TLC showed that ethyl acetate extracts mainly contained labelled glycocholic acid The butanol extracts contained several other labelled conjugates in addition to labelled taurocholic acid The isotope in the ethyl acetate extract ranged from 32 to 62 m EB associated with cholestasis and from 13 to 42 in EB not complicated by cholestasis (Table 2)

After solvolysis hydrolysis and methylation of urinary bile acids TLC analysis invariably showed one major compound with the mobility of methyl cholate Small amounts of bile acids whose TLC mobility was identical with that of methylchenodeoxycholate were additional findings in the urinary extracts from some of the patients Compounds with the same TLC mobilities as methylesters of monohydroxy

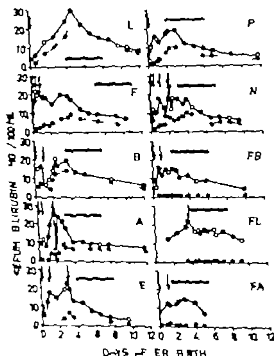


Fig 1 Serum bilirubin concentrations total (○—○) and conjugated (●—●) in infants with erythroblastosis. Arrows point toward exchange transfusions Time of isotope study (—)

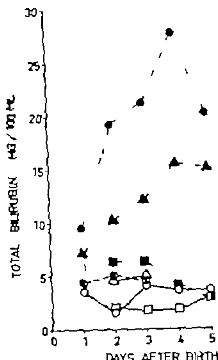


Fig 2 Total serum bilirubin concentrations in the seven normal newborn infants Normal values $M \pm 2$ SD according to Hsia G Y Y et al (3) (shadowed area) Symbols Infant P (□) F (■) W (O) K (⊙) L (Δ) S (▲) N (●)

and dihydroxycholanoic acids and compounds with TLC mobilities midway between those of the latter compounds were consistently found in the urinary extracts Autoradiography revealed the presence of one major labelled compound with the TLC mobility of methyl cholate Trace amounts of labelled compounds which were more hydrophilic than cholic acid were detected in all patients

Urinary excretion of cholic and chenodeoxycholic acids

In the 7 patients with EB associated with cholestasis the daily excretion of cholic acid ranged from 4 5 to 128 3 μmol (Table 2) Five of these patients also excreted chenodeoxycholic acid but in much smaller amounts Contrary to the 7 cases of EB associated with cholestasis the daily excretion of cholic acid was much lower in the absence of this complication ranging from 0 4 to 0 9 μmol

Table 1 *Clinical data in the 10 infants with erythroblastosis*

Patient	Sex	Gestational age (weeks)	Birth weight (g)	Maternal antibody titers of anti Rh_0	Hemoglobin in cord blood (g/100 ml)	Remarks
L	♀	37	2 460	Albumin 1/2 Papain 1/2048	4 6	Respirator 2nd–3rd day of life because of pulmonary haemorrhage Transfusion of fresh blood
F	o	38	3 210	Albumin 1/32 Papain 1/4096	6 6	Cesarean section
B	o	38	3 490	Albumin 1/64 Papain 1/512	5 7	
Å	o	35	3 260	Albumin 1/8 Papain 1/128	7 0	Cesarean section Hydrops Ampicillin treatment from 4th to 18th day of life
E	o	39	3 540	Albumin 1/4 Papain 1/256	6 3	Ampicillin treatment from 3rd to 14th day of life
P	♂	36	3 060	Albumin 0 Papain 1/32	7 6	
N	♂	36	2 440	Albumin 1/16 Papain 1/4096	5 8	
F B	o	36	2 650	Albumin 1/2 Papain 1/256	7 9	
F L	♀	38	2 910	Albumin 1/1 Papain 1/16	n d *	
F Å	♀	36	2 [illegible]00	Albumin 1/8 Papain 1/512	8 1	Cesarean section

* Not determined

during the sampling period If the stools were loose the isotope was extracted from the diapers and faeces together As some urine may be passed into the diapers the latter were divided into two groups one including diapers which contained only urine and the other including the diapers containing both urine and faeces Urine faeces and diapers were extracted as previously described and the isotope determined by liquid scintillation technique (5) Ethyl acetate and butanol extracts of urine were analyzed for free and conjugated bile acids by thin layer chromatography (TLC) Radioactive spots were located by autoradiography (5) Urine 30 ml was passed through an Amberlite XAD-2 column and the bile acids eluted with methanol (5) The residue was subjected to solvolysis alkaline hydrolysis and methylation and the final extract was analysed for unconjugated bile acids by TLC Cholic and chenodeoxycholic acids were determined by gas liquid chromatography (GLC) (5)

RESULTS

Infants with Erythroblastosis Foetalis

Isotope excretion

Table 2 gives the recoveries of the administered cholic acid 24 ^{14}C in the urine faeces and in diapers containing only urine and in diapers containing both urine and faeces Only in 4 infants was it possible to separate urine and faeces almost completely In these cases less than 5° of the administered isotope was recovered in the diapers containing both urine and faeces

In infants with EB 33–96 of the isotope was excreted during the 4 days following the injection of cholic acid 24 ^{14}C In the cases of EB associated with cholestasis most of the isotope was recovered in the urine The faeces contained 1–31 of the administered isotope In two cases of EB not complicated by cholestasis similar excretion patterns were observed but patient F A (Table 2) excreted a greater amount of the isotope in the faeces than in the urine The cumulative excretion of isotope was essentially the same irrespective of whether EB was associated with cholestasis (Fig 3 A) or not (Fig 3 B)

Nature of urinary bile acids

TLC analysis showed that small amounts of labelled cholic acid were often present in the

Table 3 *Clinical data and bile acid excretion in 7 newborns used as controls*

Infant	Gestational age (weeks)	Birth weight (g)	Sampling period (days after birth)	Urinary excretion of cholic (C) and chenodeoxycholic (CD) acids per 24 hours			
				C µmol	CD µmol	C+CD µmol	C+CD (µmol) / Creatinine (mg)
P	40	4 020	0–5	0.10	0.03	0.13	0.008
F	40	4 770	0–5	0.32	<0.01	0.32	0.023
W	39	2 980	0–5	0.68	<0.01	0.68	0.037
K	40	3 290	0–3	<0.01	<0.01	<0.01	—
L	40	3 530	0–3	<0.01	<0.01	<0.01	—
S	40	3 370	1–4	0.16	0.02	0.18	0.006
N	37	2 970	1–5	0.16	0.08	0.24	0.016

DISCUSSION

In EB the isotope excretion pattern indicated that the excretion of cholic acid 24-^{14}C to the intestine was suppressed. In 4 of the cases of EB associated with cholestasis the ratio of urinary excretion to faecal excretion was identical with that observed in intrahepatic cholestasis of infancy (5). However, the isotope was usually injected after the serum bilirubin concentration had reached its peak. Previous studies of the excretion of cholic acid 24-^{14}C in infants with intrahepatic cholestasis (5, 6) and the present investigation have shown that cholestasis may cause a decrease in both the excretion of bile acids and conjugated bilirubin to the intestine irrespective of the pathogenesis of the liver damage. In the absence of cholestasis the isotope excretion indicated that the hepatic excretion of cholic acid 24-^{14}C was impaired in EB because the urine was found to contain great amounts of conjugated labelled bile acids. However, the disturbance of the bile acid excretion seemed to be less severe in the absence of increased serum conjugated bilirubin.

All infants with EB excreted bile acids in the urine. Five infants excreted only cholic acid. The high ratio of cholic to chenodeoxycholic acid observed in the urine was probably due to cholic acid being the dominant primary bile acid during the first week of life (1). The urinary excretion of cholic and chenodeoxycholic acids in EB associated with cholestasis was as high as that which was observed in infants with intrahepatic cholestasis or extrahepatic biliary atresia (5). However, in the absence of cholestasis the urinary bile acid excretion was much lower. There was no noteworthy difference between three cases of EB associated with cholestasis (E, P and N) and the three cases not associated with cholestasis with respect to the excretion patterns of cholic acid 24-^{14}C. The discrepancy between the quantitative urinary excretion of bile acids and the isotope excretion in these cases seems to indicate that the synthesis of bile acids in the liver is greater in EB associated with cholestasis than in the absence of this complication. The determination of bile acids in the urine showed that 3 of the 5 infants with physiological jaundice and the 2 infants with hyperbilirubinaemia excreted practically the same amounts of bile acids as did the infants with EB not associated with cholestasis. If not complicated by cholestasis, EB alone did not cause an appreciable change in the excretion pattern of bile acids.

SUMMARY

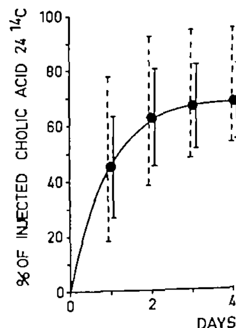
The excretion pattern of intramuscularly injected cholic acid 24-^{14}C was studied for 4 days after the injection in 10 cases of erythroblastosis (EB). Seven patients with EB and raised serum conjugated bilirubin excreted 36–63 of the injected isotope in the urine

Table 2 *Bile acid excretion in the 10 infants with erythroblastosis (EB)*

Patient	Isotope excretion of administered cholic acid 24 ^{14}C: Urine (Diapers)	Faeces	Total (Diapers containing urine and faeces)	Urinary labelled metabolites extractable with ethyl acetate (glycine conjugates)	Urinary excretion of cholic (C) and chenodeoxycholic (CD) acids per 24 hours: C µmol	CD µmol	C+CD µmol	C+CD (µmol) / Creatinine (mg)
EB associated with cholestasis								
L	51 (23)	<1	58 (7)	44	128 3	4 0	132 3	5 75
F	46 (14)	1	56 (8)	32	4 5	0 2	4 8	0 34
B	44 (1)	7	65 (14)	48	18 3	3 2	21 4	0 56
Å	41 (<1)	13	54 (0)	47	7 4	<0 1	7 4	0 26
E	44 (4)	24	79 (11)	51	13 3	<0 1	13 3	0 43
P	36 (<1)	25	75 (14)	48	7 5	1 4	8 9	0 23
N	63 (<1)	31	96 (1)	62	17 9	2 1	20 0	0 67
EB not associated with cholestasis								
F B	33 (<1)	29	65 (3)	42	0 9	<0 1	0 9	0 04
F L	20 (1)	11	33 (1)	13	0 9	<0 1	0 9	0 04
F Å	15 (<1)	42	71 (15)	20	0 4	<0 1	0 4	0 02

Normal Newborn Infants with Physiological Jaundice and Newborn Infants with Hyperbilirubinaemia

After solvolysis hydrolysis and methylation TLC of the samples of urine revealed the presence of a compound with the mobility of methylcholate in 5 of the 7 controls An additional finding was a compound with the TLC mobility of methylchenodeoxycholate in 3 of the 5 controls The daily excretion of cholic acid ranged from less than 0 01 to 0 7 µmol (Table 3)



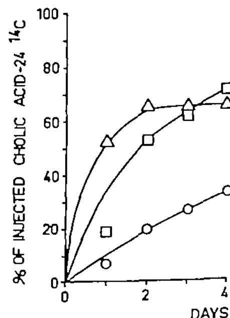


Fig 3 Cumulative excretion of total isotope in urine and faeces after intramuscular injection of cholic acid 24 ^{14}C in cases of erythroblastosis (*A*) Cases associated with cholestasis Mean (●) range ( ) and ±SD (—) (*B*) Cases not associated with cholestasis. Symbols Infant F B (Δ) F L (O) and F Å (□)

α_1 FETOPROTEIN CONCENTRATION IN CORD SERUM AS A PARAMETER FOR GESTATIONAL AGE

B NØRGAARD PEDERSEN

From the Department of Clinical Biochemistry Rigshospitalet University of Copenhagen Denmark

A distinct α globulin in the serum from the human fetus was first demonstrated by Bergstrand & Czar (2) by means of paper electrophoresis This protein called α_1 fetoprotein shows a maximum concentration in the fetus around the 14th week of gestational age and then gradually decreases with increasing gestational age (3 10 14 15 28) It is produced by the fetal liver and yolk sac (11) Reappearence of α_1 fetoprotein in significant amounts in adult serum occurs in primary liver cancer and the concentration of α_1 fetoprotein in the serum can be used as a diagnostic parameter in this disease (1 12 23) The concentration in normal adult sera is very low and only measurable by radioimmunoassay technique (22 24 25) While the connection between α_1 fetoprotein and primary liver cancer has been dealt with very intensively in litterature in the last few years (for review see Abelev (1)) only small attention has been given the α_1 fetoprotein as a maturity parameter (3 10 14 15)

The purpose of the present investigation is therefore firstly to describe a simple technique for maturity studies in newborn infants based upon immunoelectrophoretical quantitation of α_1 fetoprotein in cord serum secondly to evaluate the correlation statistically and to presents confidence limits for estimation of gestational age by means of the α_1 fetoprotein concentration and finally to compare the present correlation with the correlation between gestational age and some external characteristics such as birth weight crown–heel length and head circumference

MATERIAL

In order to ensure reliable information about gestational age the following criteria were used for selection of the material The information was collected from the hospital records An exact date for the beginning of the LMP was claimed Regular and normal menstrual cycles 28±3 days should be present before pregnancy and no bleeding must occur during the first trimester After oral contraception at least one normal menstruation should have occurred Perception of the first fetal movements should be within the normal limits for primipara (18th to 20th week) and multipara (16th to 20th week) Information about gynaecological examination and growth of uterus in the early antenatal period should agree with gestational age Furthermore pathological pregnancies such as diabetes mellitus toxaemia and immunization are not included in the material

Investigated material

Cord blood samples were collected over a 5 month period from 407 patients just after delivery The obtained cord sera were frozen at –20 C until analysed

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Definitions and abbreviations used

Gestational age The postmenstrual age in days from the first day of the last menstrual period of the mother until the day of birth. Preterm means gestational age less than 267 days Term means gestational age between 267 and 294 days Postterm means gestational age more than 294 days. LMP is the last menstrual period SGA, AGA and LGA (Small Appropriate and Large for Gestational Age) are infants with birth weight below within or above the normal limits (10th 90th percentile) for gestational age according to standard weight curves (17 26 27 29)

whereas the amounts of isotope in the faeces varied greatly In 3 cases without raised serum conjugated bilirubin less isotope was recovered in the urine and always more than 10% of injected isotope was recovered in the faeces Cholic acid 24-^{14}C was excreted essentially unchanged in all cases but in conjugated form

In all cases of EB the urine was found to contain bile acids chiefly cholic acid The infants with EB associated with cholestasis excreted 4 8–132 3 μmol of these acids per day the corresponding values in the absence of cholestasis being 0 4–0 9 μmol per day In the infants with physiological jaundice the excretion ranged from less than 0 01 to 0 7 μmol per day the correspondign values in the 2 patients with hyperbilirubinaemia were about 0 2 μmol per day

The infants with EB associataed with cholestasis were found to excrete as large amounts of bile acids in the urine as the infants with intrahepatic cholestasis These findings strongly suggest that increased serum conjugated bilirubin irrespective of the pathogenesis of the liver damage is associated with an impaired bile acid excretion to the intestine EB without increased serum conjugated bilirubin did not seem to alter the bile acid metabolism since the urinary excretion of cholic acid and chenodeoxycholic acid in these cases was practically the same as in jaundiced newborn infants

ACKNOWLEDGEMENTS

We wish to express our special gratitude to our colleagues and nurses on the staff of the neonatal ward at the Department of Pediatrics Karolinska sjukhuset, Stockholm where the infants with EB were treated We are indebted to Mrs Iris Samuelsson and Miss Ann Christin Hoff for skilful technical assistance

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α_1 FETOPROTEIN CONCENTRATION IN CORD SERUM AS A PARAMETER FOR GESTATIONAL AGE

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A distinct α globulin in the serum from the human fetus was first demonstrated by Bergstrand & Czar (2) by means of paper electrophoresis. This protein, called α_1 fetoprotein[1], shows a maximum concentration in the fetus around the 14th week of gestational age and then gradually decreases with increasing gestational age (3, 10, 14, 15, 28). It is produced by the fetal liver and yolk sac (11). Reappearence of α_1 fetoprotein in significant amounts in adult serum occurs in primary liver cancer and the concentration of α_1 fetoprotein in the serum can be used as a diagnostic parameter in this disease (1, 12, 23). The concentration in normal adult sera is very low and only measurable by radioimmunoassay technique (22, 24, 25). While the connection between α_1 fetoprotein and primary liver cancer has been dealt with very intensively in litterature in the last few years (for review see Abelev (1)) only small attention has been given the α_1 fetoprotein as a maturity parameter (3, 10, 14, 15).

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Gestational age: The postmenstrual age in days from the first day of the last menstrual period of the mother until the day of birth. Preterm means gestational age less than 267 days. Term means gestational age between 267 and 294 days. Postterm means gestational age more than 294 days. LMP is the last menstrual period. SGA, AGA and LGA (Small, Appropriate and Large for Gestational Age) are infants with birth weight below, within or above the normal limits (10th–90th percentile) for gestational age according to standard weight curves (17, 26, 27, 29).

The purpose of the present investigation is therefore firstly to describe a simple technique for maturity studies in newborn infants based upon immunoelectrophoretical quantitation of α_1 fetoprotein in cord serum, secondly to evaluate the correlation statistically and to presents confidence limits for estimation of gestational age by means of the α_1 fetoprotein concentration and finally to compare the present correlation with the correlation between gestational age and some external characteristics such as birth weight, crown–heel length and head circumference.

MATERIAL

In order to ensure reliable information about gestational age the following criteria were used for selection of the material. The information was collected from the hospital records. An exact date for the beginning of the LMP was claimed. Regular and normal menstrual cycles 28±3 days should be present before pregnancy and no bleeding must occur during the first trimester. After oral contraception at least one normal menstruation should have occurred. Perception of the first fetal movements should be within the normal limits for primipara (18th to 20th week) and multipara (16th to 20th week). Information about gynaecological examination and growth of uterus in the early antenatal period should agree with gestational age. Furthermore pathological pregnancies such as diabetes mellitus, toxaemia and immunization are not included in the material.

Investigated material

Cord blood samples were collected over a 5 month period from 407 patients just after delivery. The obtained cord sera were frozen at −20 C until analysed

Table 1 *The investigated material grouped according to sex and gestational age groups*

Gestational age (days)	Total material	Boys	Girls
< 225	5	4	1
225–238	11	5	6
239–252	20	15	5
253–266	29	17	12
267–280	84	46	38
281–294	95	47	48
> 294	14	8	6
Total	258	142	116

for the α_1 fetoprotein concentration After quantitation information was collected from the hospital records Only 258 infants namely 116 girls and 142 boys fulfilled the above mentioned criteria Table 1 shows the material classified according to sex and gestational age using 2 weeks interval 21 infants (LGA) had a birth weight above the 90th percentile 198 (AGA) between the 10th and 90th percentile and 39 (SGA) below the 10th percentile The classification was performed according to the Swedish standard curves for boys and girls (26) Besides the above mentioned material sera were collected from 7 twins and in 20 infants at different times after birth No significant difference in the α fetoprotein concentration was found in each pair of twins

METHODS

The birth weight the crown–heel length and the largest head circumference were measured by a midwife just after delivery The weight was recorded within 10 g and crown–heel length and head circumference was recorded within 0 5 cm

Quantitation of α_1 foetoprotein was carried out by rocket immunoelectrophoresis as previous described (16 21) Antigen α foetoprotein standard 0 5 ml 270 mg/l (Behringwerke AG Marburg) Antibody rabbit immunoglobulins to human α foetoprotein (Dakopatts Copenhagen) Procedure Immunoelectrophoresis is carried out in a 1 mm thick layer of 1 agarose gel containing anti-α foetoprotein serum (1 v/v) with 3 V/cm for 12–18 hours Dilution of standards with barbitone buffer pH 8 6 (v/v) 1+1 1+3 1+7 and 1+15 The cord sera samples are either used undiluted or diluted 1+9 Exactly 3 μl is filled into the holes before electrophoresis The very sensitive staining with Coomassie Brilliant Blue is used The quantitation of α foetoprotein in cord sera can be carried out with high voltage electrophoresis and the results are then available within 2–3 hours after birth

Statistical methods

α_1 foetoprotein birth weight crown–heel length and head circumference were each correlated with gestational age and with each other using a linear regression analysis computer program (NEUCC, Northern Europe University Computing Center) In order to test whether the correlation between α foetoprotein was nonlinear different exponential regression analysis was used Furthermore the log values of the various parameters was tested to see if improvement of the correlation could be obtained The correlation coefficients the variance of the material around the regression line and the variance for the slope of the regression line was calculated From these two variance values the 95 confidence limits for estimation of gestational age (y) was calculated for each maturity parameter (x) Multiple linear regression analysis was used in order to obtain the best equation for estimation of gestational age

RESULTS

In all cord sera a visible immunoprecipitate suitable for quantitation was obtained The correlation between the α_1 foetoprotein concentration in cord sera and gestational age is shown in Fig 1 for boys and girls respectively The coefficient of correlation was found to be −0 89 and −0 88 for all boys and all girls respectively For boys and girls of 280 days of gestational age or less the coefficient was significantly higher namely −0 92 for both This was a higher degree of correlation than found for birth weight crown–heel length and head circumference (see Table 2) The correlation between birth weight and gestational age for boys and girls is shown in Fig 2 A highly significant difference ($p < 0.01$) is found between the α_1 foetoprotein concentration in the different gestational age groups (Table I) except for 281–294 days against > 294 days where the difference is less marked ($p < 0.05$) It means that a significant difference is found between different groups of preterm infants at 2 week intervals but a less pronounced difference between term and post term infants

The correlation between α_1 foetoprotein and birth weight was less marked $r = -0.60$ and $r = -0.59$ No significant difference in α_1 foetoprotein concentration in cord serum was found between SGA and AGA groups and between LGA and AGA groups with the same gestational age Application of stepwise multiple regression

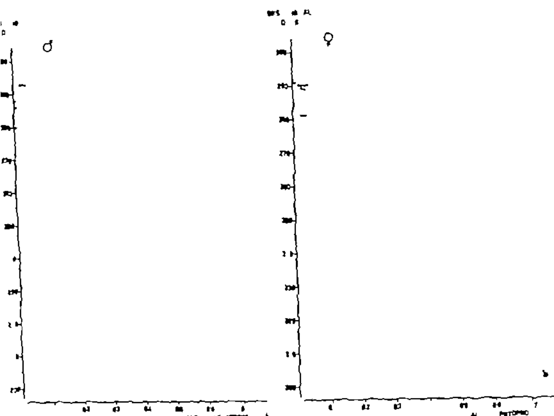


Fig. 1. The correlation between α_1 fetoprotein (g/l) and gestational age (days) for boys (a) and girls (b). The formula for th regression lines are $y = 290 - 132\,x$ for boys and $y = 289 - 1?8\,x$ for girls. Infants weighing less than 2 500 g = x.

birth weight, crown–heel length and head circumference did not improve the correlations. The postnatal changes in α_1 foetoprotein concentration in serum from 20 newborn infants show varying degree of decrease. In some sera no change in concentration is observed during the first 24 hours of life, while in other sera a marked fall in concentration is found. Even during the first few hours of life a marked fall up to 0.1 g/l has been measured in some newborn infants. The calculated mean half time for the decrease in α_1 foetoprotein concentration after birth was in 20 newborn infants 5 days.

Table 2. *Linear regression equations for all infants as well as for infants < 280 days*

y (days of gestation) and x (α_1 foetoprotein, birth weight, crown-heel length and head circumference) and the corresponding values for coefficients of correlation ( ), the variance (s^2) and 95 confidence limits for estimating the gestational age

	All infants (n 238)				Infants of gestational age < 280 (n = 149)			
Y variable	Equation no. 1		s^2	Limits in days	Equation no. 2		s^2	Limits in days
α_1-fetoprotein mg/l	$y = 290 - 130\,x$	−0.89	56	±16	$y = 282 - 107\,x$	−0.92	33	±13
Birth weight g	$y = 216 \; 0.018\,x$	0.69	141	±?6	$y = 213 - 0.017\,x$	0 4	99	±22
Crown-heel length cm	$y \; 65 + 4.18\,x$	0.74	1 4	±25	$y = 80 + 3.79\,x$	0.77	87	±21
Head circumference cm	$y = 11 \; 4.71\,x$	0.61	172	± 9	$y = 104 + 4.75\,x$	0.69	113	±24

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The correlation between α_1 foetoprotein and birth weight was less marked $r=-0\ 60$ and $r=-0\ 59$ No significant difference in α_1 foetoprotein concentration in cord serum was found between SGA and AGA groups and between LGA and AGA groups with the same gestational age Application of stepwise multiple regression procedure for α_1 foetoprotein

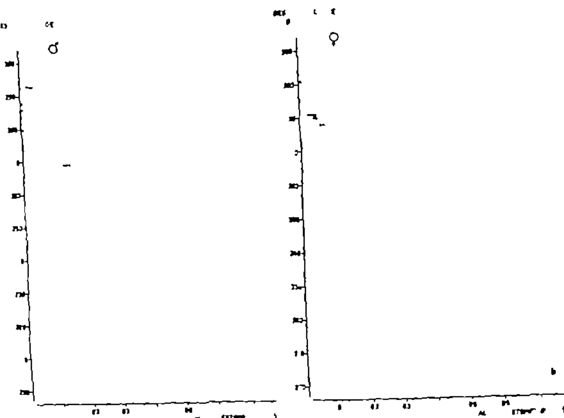


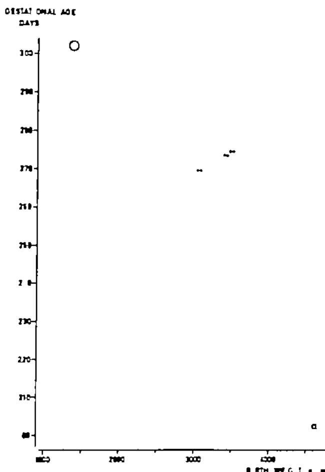
Fig 1 The correlation between fetoprotein (g/l) and gestational age (days) for boys (a) and girls (b) The formula for th regression lines are $y = 290 - 132\,x$ for boys and $y = 289 - 1\ 8\,x$ for girls Infants weighin less than 2 500 g = x

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Table 2 *Linear regression equations for all infants as well as for infants ≤280 days*

y (days of gestation) and *x* (foetoprotein birth weight crown–heel length and head circumference) and the corre sponding values for coefficients of correlation ( ) the variance (s^2) and 95 confidence limits for estimating the gesta tional age

	All infants (n 258)				Infants of gestational age ≤280 (n = 149)			
Y variable	Equation no 1	r_{xy}	s^2	Limits in days	Equation no 2	r_{xy}	s^2	Limits in days
fetoprotein mg/l	y 290 130 x	0 89	56	±16	$y = 282 - 107\,x$	−0 92	33	±13
Birth weight g	y 16 0 018 x	0 69	141	±26	$y = 213 - 0\ 017\,x$	0 74	99	±—
Crown–heel length cm	y 63 + 4 18 x	0 74	124	±25	$y = 80 + 3\ 79\,x$	0 77	87	±21
Head circumference cm	$y = 11$ 4 71 x	0 61	172	±29	$y = 104 + 4\ 75\,x$	0 69	113	±24



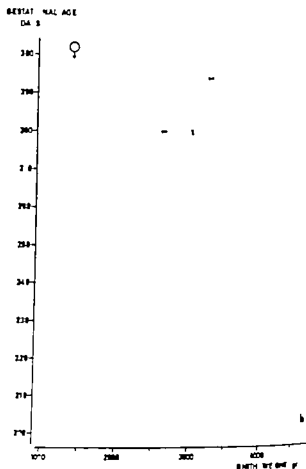


Fig 2 The correlation between birth weight (g) and gestational age (days) for boys (a) and girls (b) The formula for the regression lines are $y = 214 + 0.018\,x$ for boys and $y = 221 + 0.017\,x$ for girls

DISCUSSION

Postnatal estimation of gestational age has mainly been carried out by means of physical measurements external characteristics and neurological tests (4 6 7 8 9 13 18 19 29) Usually a total maturity score is calculated giving a 95% confidence limits for prediction of gestational age from ±2 to ±4 weeks (4 6 9) Also laboratory tests such as hemoglobin F concentration and carbonic anhydrase activity in cord blood have been used giving confidence limits about ±3 weeks (5 20) In the present study α_1 foetoprotein has been shown to be a far better indicator of gestational age than anthropometric characteristics such as birth weight, crown–heel length and head circumference The concentration of α_1 foetoprotein at different gestational ages found in this study is in agreement with the concentration reported by others (3 10 14) The calculated 95% confidence limits for estimating gestational age by means of α_1 foetoprotein concentration in cord blood was ±16 days The limits reported by Bergstrand et al (3) was ±4.3 weeks This great difference may be due to a less precisely defined material in the study by Bergstrand et al and to differences in the time for collection of samples In this study 149 out of 407 infants were excluded nearly always because of unreliable information about gestational age A marked postnatal fall in the serum α_1 foetoprotein concentration in newborn infants may be observed as also described by Karlsson et al (14) To obtain the close correlation between α_1 foetoprotein concentration and gestational age as in the present study cord sera should be used for quantitation The collection of cord sera is easy and well defined and may be done without special experience

When comparing different gestational age groups a highly significant difference in α_1 foetoprotein concentration was found in all gestational age groups of Table 1 except for 281–294 days against >294 days The same conclusion could be predicted from Fig 1 Therefore the confidence limits for estimating gestational age in infants ≤280 days was only ±13 days As seen from Table 2 the confidence limits for external characteristics are also smaller for infant ≤280 days The question is whether the post term infants represent a group or not Each pair of twins showed nearly the same α_1 foetoprotein concentration They are expected to do so if the α_1 foetoprotein concentration is dependent of gestational age only As shown by Bergstrand et al (3) no difference in α_1 foetoprotein concentration was found in SGA AGA and LGA with the same gestational age The correlation between α_1 foetoprotein concentration in cord blood and birth weight is also less ($r = -0.60$) than between α_1 foetoprotein and gestational age ($r = -0.89$)

In conclusion laboratory assessment of gestational age by means of α_1 foetoprotein concentration in umbilical cord blood represents a valuable tool in testing the degree of development of the newborn infant The method is easy and reliable and is *without* any risk for the mother and the newborn Whether it is unaltered in pathological pregnancies remains to be shown

SUMMARY

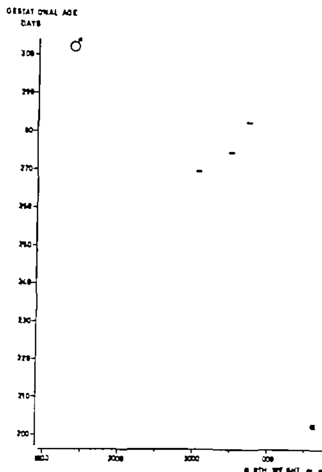
Quantitation of human α_1 foetoprotein by Laurell immunoelectrophoresis in cord serum from 258 newborn infants with a gestational age between 306 and 210 days showed a close negative correlation with gestational age ($r = -0.89$) The correlation between α_1 foetoprotein and birth weight was less pronounced ($r = -0.60$) No difference in the concentration of α_1 foetoprotein was found in Small for Gestational Age (SGA) Appropriate for Gestational Age (AGA) and Large for Gestational Age (LGA) infants with the same gestational age From a given α_1 foetoprotein concentration in cord blood the confidence limits for prediction of gestational age is ±16 days In 149 infants with a gestational age ≤280 days the correlation between α_1 foetoprotein and gestational age was even better ($r = -0.92$)

ACKNOWLEDGEMENTS

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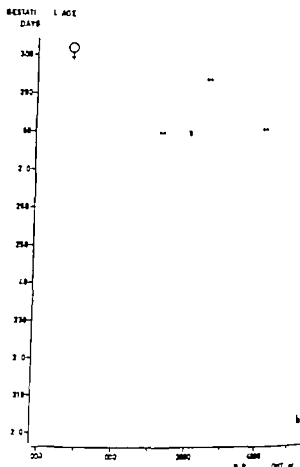


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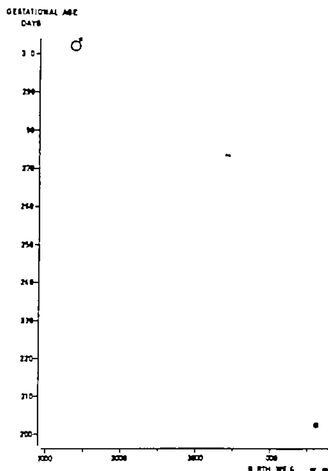
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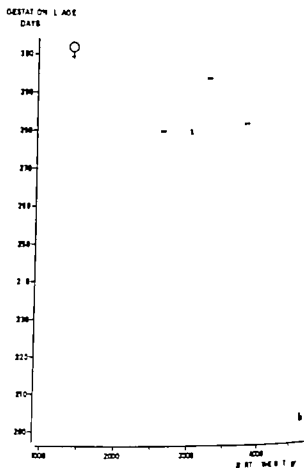


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Acta Pædiat Scand 62 173–175 1973

STORAGE IRON IN FOETAL LIVERS

L L CHANG

From the Department of Physiology University of Singapore Singapore

Storage iron comprising approximately 25 of the total iron in the body is an important fraction since it represents a reserve from which the body may mobilise iron for erythropoiesis The chief sites in the body for iron storage are the liver spleen and bone marrow Iron is stored in these sites as 2 main chemical forms ferritin and hemosiderin Ferritin is a water soluble iron protein complex which readily shows up on electron microscopy as a characteristic cluster of four dots representing iron micelles (3) It is not visible by the Prussian Blue reaction Hemosiderin on the other hand is insoluble in water and can be visualised in tissues without staining as golden yellow granules or by the Prussian Blue reaction (8) These two storage compounds are functionally related to one another and iron is mobilised from both sources when the need arises (9) though perhaps more readily from the ferritin fraction (11)

The liver is one of the main storage organs for iron and the amount of iron in foetal liver represents the iron reserve with which the child starts its life The magnitude of this iron reserve is also a reflection of the adequacy of iron nutrition in the mother because foetal parasitism is not total and maternal iron under nutrition is also shared by the foetus (10) It was thought useful for these reasons to study the liver storage iron content in stillborn babies in Singapore

MATERIAL AND METHODS

24 fresh still born babies, of both sexes, delivered at Kandang Kerbau Maternity Hospital Singapore were estimated for the total storage (non haem) iron content of the liver by the method described by Kaldor (7) Preliminary study of the method which uses o-phenanthroline as the colour reagent revealed that it specifically extracts and measures non haem iron Less than 5% of the haem iron in the tissue is estimated normally For comparison the kidneys of the babies were also estimated for their storage iron content

There were 22 Chinese and 2 Indian babies They were divided into 2 groups according to their gestational ages as calculated from the mother's menstrual history The first group comprised 12 premature babies with a mean birth weight of 1 239 8 g (±288 1) and gestational ages ranging from 28 weeks to 34 weeks The second group comprised 12 mature babies with a mean birth weight of 3 014 6 g (±375 3) and gestational ages ranging from 38 weeks to 41 weeks

RESULTS

The mean concentration of storage iron in the liver of the 24 foetuses with gestational ages ranging from 28 weeks to 41 weeks was 0 328 mg/g wet weight (±0 146) with a mean total storage iron content of 31 2 mg (±17 3) The mean concentration of storage iron in the kidneys of these foetuses was 0 033 mg/g wet weight (±0 012) with a mean total storage iron content of 0 78 mg (±0 48) The liver is by far the more important storage organ for iron There was a significant positive correlation between the storage iron content of the liver and the kidney ($r = 0\ 593$ $p < 0\ 01$)

A comparison between the group of babies born prematurely and the group born at full term showed that the storage iron content of the liver and kidney more than doubled in the last few weeks of gestation (Table 1) The concentration of storage iron rose significantly in the kidney but not in the liver

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A comparison between the group of babies born prematurely and the group born at full term showed that the storage iron content of the liver and kidney more than doubled in the last few weeks of gestation (Table 1) The concentration of storage iron rose significantly in the kidney but not in the liver

Table 1 *A comparison between the mean concentration and mean total storage iron in the livers and kidneys of premature and mature stillborn infants*

		Mean values ±1 S D						
Group	No of cases	Birth weight (g)	Liver weight (g)	Conc of storage iron in liver (mg/g wet weight)	Total storage iron in liver (mg)	Kidney weight (g)	Conc of storage iron in kidney (mg/g wet weight)	Total storage iron in kidney
Premature infants	12	1239 8 ±288 1	61 8 ±20 7	0 274 ±0 092	20 2 ±11 2	14 6 ±5 8	0 027 ±0 012	0 43 ±0 22
Mature infants	12	3 014 6 ±375 3	115 4 ±24 0	0 383 ±0 172	42 2 ±15 4	29 6 ±5 8	0 038 ±0 010	1 13 ±0 41
Test of significance		$t=12\ 99$ $p<0\ 001$	$t=5\ 848$ $p<0\ 001$	$t=1\ 938$ not signif	$t=4\ 005$ $p<0\ 001$	$t=6\ 045$ $p<0\ 001$	$t=2\ 249$ $p<0\ 05$	$t=4\ 995$ $p<0\ 001$

Table 2 relates the storage iron content of the liver and kidney with both birth weight and gestational age There was a significant positive correlation between these parameters The bigger the infant and the more advanced the gestational age the higher the amount of storage iron in both liver and kidney

DISCUSSION

The concentration of storage iron in the foetal liver found in this study is similar to that of Buchanan (2) in Rhodesia Wainwright (as quoted from Buchanan 2) in South Africa and Bruckman & Zondek (1) in Palestine This concentration of liver storage iron exceeds that found in healthy adult males (11) The similarity of these results derived from different countries is striking It probably means that mechanisms exist to prevent foetal iron over load Rhodesian African foetuses do not reflect the common occurrence of excess storage iron seen in their mothers (2) The converse however is not true Foetuses of comparable gestational age and birth weight vary considerably in their liver storage iron content This could be due to maternal iron deficiency Clinical studies on the stainable bone marrow of women show that a large majority of women enter pregnancy with depleted iron stores (4)

Table 2 *Test of correlation between storage iron measurements in the foetal liver and kidney with birth weight and gestational age of the foetuses*

Correlation between	Correlation coefficient	Significance
Birthweight of foetus and total storage iron in the liver	$r=0\ 740$	$p<0\ 001$
Gestational age of foetus and total storage iron in the liver	$r=0\ 648$	$p<0\ 001$
Birthweight of foetus and total storage iron in the kidney	$r=0\ 783$	$p<0\ 001$
Gestational age of foetus and total storage iron in the kidney	$r=0\ 668$	$p<0\ 001$

The significant positive correlation between the total storage iron in the liver and kidney with both body weight and gestational age reflects a similar finding between the total body iron and body weight (12) and between total body iron and gestational age (5) The total storage iron in the liver and kidney rises steeply in the last weeks of pregnancy Infants born before the 36th week of gestation on the average have less than half the amount of reserve iron in their livers compared with infants born later The tendency for premature infants to develop iron deficiency anemia more often and at an earlier stage in infancy than mature infants is well known (13 14 15) This has been attributed to factors like their greater rate of growth with its greater demand for iron their sole dependence on milk with its low iron content and their smaller stores of reserve iron These iron stores not only reside

in the main storage organs like the liver spleen and bone marrow but also in the circulating hemoglobin mass Quantitatively the iron reserve in the form of hemoglobin exceeds that provided for in the storage depots (6) The storage iron component however provides a ready source of iron mobilisable for erythropoiesis whilst iron from hemoglobin can only be utilised after its release from senescent red cells The importance of such stores being adequate at birth both in mature and premature infants should not be ignored

Iron supplementation should therefore be practised more often in infants when the situation demands

SUMMARY

Storage iron was estimated in the livers and kidneys of 12 premature and 12 mature still born infants In both organs the total storage iron content more than doubled during the last weeks of gestation There was a significant positive correlation between the level of storage iron in these organs and the birth weight and gestational age of the infant

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DYNAMIC PRESSURE VOLUME RELATIONSHIP OF THE LUNG AND POSITION IN HEALTHY NEONATES

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General nursing of the newborn in prone position has been practised in North America and the Scandinavian countries for a long time However this position—because of lingering doubt as to its efficiency—has not been fully accepted in other parts of Europe Gleiss (3) discussed the possible advantages of prone over supine position of the infant and referred to theories of Mau (4) and Reisetbauer (5) Reisetbauer suggested that nursing in prone position apart from other advantages (Fig 1) might offer better respiratory mechanical conditions To test the latter hypothesis dynamic pressure volume diagrams of the lung as one of the possible parameters of lung mechanics were registered in 17 healthy neonates in various positions

METHOD

Dynamic pressure volume loops were made by relating flow integrated volume to transpulmonary pressure The latter was measured as the difference between oesophageal pressure and pressure at the nostrils To measure oesophageal pressure an infant feeding tube provided at the end with a latex balloon was introduced orally into the lower 1/3 to 2/5 part of the oesophagus Flow was measured with a Fleisch pneumotachograph No 00 connected to a nasal mask The nasal mask modified after Buck & McCormack (2) was moulded to fit by means of relining material and was fixed to the infant s nose with surgical cement The method has been described elsewhere in detail (7) The flow integrated volume and transpulmonary pressure signals were transmitted to Y and X axis of a cathode ray tube respectively The loops were photographed from the screen Attention was given to the slope of the line connecting the beginning and the end of inspiration of V/P loops which in limited range of lung volume represented compliance of the lungs and to width of the loops which represented non-elastic resistance

RESULTS

At the beginning of the investigation changes in V/P loops in both prone and supine positions were often noted but proved to be not always reproducible This prompted our attention to the position of the head in relation to the body In both positions rotation of the head decreased the slope of V/P loops Fig 2 shows the changes in the loops obtained from a newborn of 2 660 g in supine position when the head was turned to one side

With the head in median position 45 degrees flexion resp 45 degrees extension V/P loops were derived from a newborn of 2 840 g There was no difference between the loops obtained in supine position with the head in normal position and with extension of the head but the slope of the loops with 45 degrees flexion of the head decreased (Fig 3) Fig 4 shows that lying in prone position with the head in median position (a newborn of 2 500 g) the slope of the loops was markedly decreased and their width increased when the head was in flexion position and returned to normal when the head was in slight extension (upright) position

When considering the group of newborn infants studied a significant change was ob

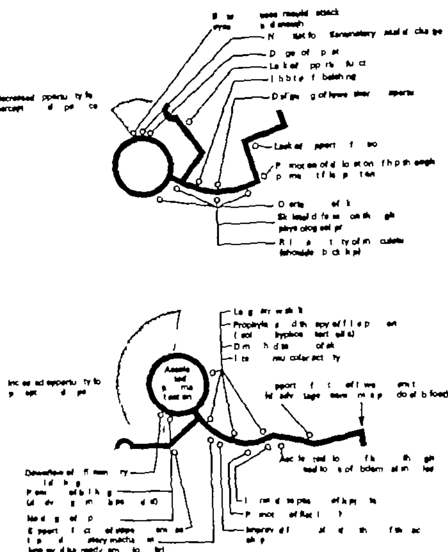


Fig 1 Disadvantages of supine position and advantages of prone position of infant (after Reinelbauer & Glenn)

served between rotation and median position of the head but there was no *significant* change in supine and prone position with the head in median position (Table 1 and Fig 5) During the measurements the respiratory rates in all cases remained within the range of 30–40 per minute

DISCUSSION

By flexion and rotation of the head the slope of the dynamic V/P loops was decreased and the width of the loops increased The latter might be due to an increase of non elastic resistance caused by deformation and narrowing of the upper airways Similar changes were also observed in experiments on dogs (6) Changes in the upper airways *with different* positions of the head in infancy were roentgenologically described elsewhere (1)

In the prone position with the head in median position compared with supine position the slope of the loops in some cases has

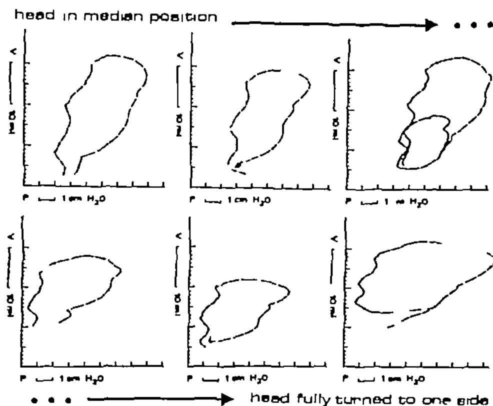


Fig 2 Effect of rotation of the head on V/P loops (case 10)

Fig 3 Effect of normal median position extension and flexion of the head in supine position on V/P loops (case 13)

Fig. 4. Effect of normal median position, flexion and upright position of the head in prone position on V/P loops (case 3).

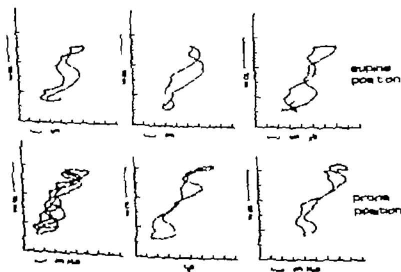


Fig. 5. Effect of normal median position of the head in supine position and in prone position on V/P loops (case 14).

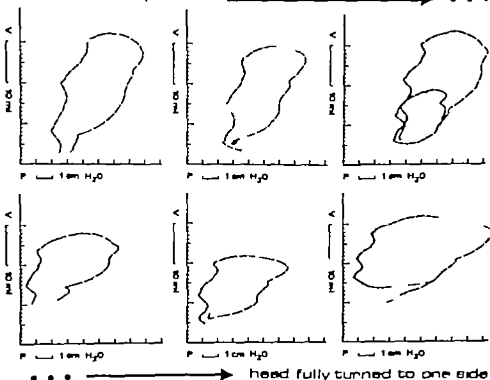


Fig 2 Effect of rotation of the head on V/P loops (case 10)

Fig 3 Effect of normal median position extension and flexion of the head in supine position on V/P loops (case 13)

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PHYSICAL EXAMINATION OF FOUR YEAR OLD CHILDREN

LENNART KÖHLER

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Child health services have been traditionally centred around the medical evaluation of individual children performed by a physician To day however more and more emphasis is laid on various screening procedures performed by non physician personnel In a study of 4-year old children intended to bridge the gap of efficient health control of children between infancy and school age traditional medical evaluation as well as screening methods were included (dental examination auditory screening vision screening bacteriuria screening psychological examination) Reports from these studies are published elsewhere (17 21 22 23 24)

The principal aim of the present part of the investigation was to study the physical health problems in an unselected population of pre school children especially in those not previously detected or cared for

MATERIAL

Since 1967 general health control of 4 year-old children has been organized in the city of Lund and since 1969 in the community of Dalby in the county of Lund Names and addresses of all children at the age of 4 years living in these areas were extracted from the county population register Children living temporarily in the areas but registered elsewhere were excluded There was total of 573 4 year-old children 2 96 living in Lund 1967 1969 and 77 in Dalby 1968 1969 Altogether 2 447 children (1 boys and 1 175 girls) 95 1 participated in the study Great effort was spent in making the examination fun for the children and it was possible to get full cooperation in all but 5 boys and 8 girls (99.5) Some of the most important motor functions of these few un-cooperative children could be studied very well as they fought crawled under tables or ran away

METHODS

The children were invited to participate by a letter to their parents The invitation was accompanied by questionnaires regarding *inter alia* the child's development previous and present health problems and the family's social standard A 3 graded socio-economic grouping system widely used in Sweden was employed (6) This pays special attention to paternal occupation group I representing the highest group The records of children already under professional care were checked but otherwise the information from the parents was not confirmed from other sources Also existing records of those children who failed to appear were checked at the Department of Paediatrics which is the main centre caring for physically handicapped children in this area

The physical examination was performed at two Child Health Centres by the same paediatrician (the author) and followed a standardized scheme One of the parents generally the mother was present The examination took an average of ‚0 min The following items were included

1 *Anthropometrical measurements*

Height The child stood barefoot on the floor with his feet together and the heels buttocks and back of the head touching the wall where a scale was fixed The line between the auditory meatus and the lateral margin of the orbit was parallel to the floor A wooden headpiece was lowered until it touched the top of the head Recordings were made within 0 5 cm

Weight The child was weighed on a platform balance (Stathmos 304 AB Lindell Jonkoping Sweden) The zero setting of the balance was checked each morning Recordings were made within 0 1 kg

Head circumference The maximum circumference across the frontal bone area and the occiput was measured with a narrow metal tape Recordings were made within 0.5 cm

Table 1 *Dynamic lung compliance (ml cm⁻ H_2O) of the newborn in different lying positions*

Position of the head		Supine position					Prone position		
No	Weight (g)	Normal median	Rotation	Flexion	Extension	Normal median	Normal median	Flexion	Extension
1	2 360					8.74	6.65		
2	2 450					7.56	8.00		
3	2 500			5.95	6.50	7.02	4.91	4.48	5.84
4	2 540			3.10	10.00	9.09	5.07	3.75	5.73
5	2 550	9.00	4.86	6.18	8.57	9.09			
6	2 570	8.80	5.47						
7	2 590						7.63	7.74	6.03
8	2 600			7.77	9.28	8.72	9.24	5.23	6.96
9	2 603			6.52	7.71	8.00	7.27	7.55	8.44
10	2 660	10.00	5.12						
11	2 740	6.88	4.57				7.01	4.25	7.57
12	2 820					5.53	7.05		
13	2 840			4.85	6.40	6.89			
14	2 850					8.33	8.00		
15	3 080				8.94	9.03			
16	3 140	10.95	5.90	4.93	10.72	10.40	6.10	6.33	7.69
17	3 190			5.33	8.16	8.38	9.20	8.80	10.00
		$p < 0.001$				$p > 0.05$			

already decreased In other words prone position possibly causes a deformation of the upper airways already with the head in extension however the slope of the loops returns to normal Usually in the supine position the infant tends to lie—because of its relative round trunk and long narrow skull—on one side i e the head in relation to the body is not greatly turned away from the median line but in prone position—especially during sleep—its head is usually turned to one side which as already shown above affects the V/P loops in the sense as mentioned before In other words prone position tends to result in inhibition instead of improvement of lung mechanics We therefore have the impression that Gleiss et al did not take into account the position of the head in relation to the body when advocating a prone nursing position

SUMMARY

There is no significant difference in the dynamic V/P loops of the lung between supine position with the head in normal median position and prone position i e when there is no deformation of the upper airways In both prone and supine position with flexion or rotation of the head the slope of the V/P loops was decreased and the width of the loops increased In pulmonary function tests special attention should therefore be given to the position of the head in relation to the body

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Key words Dynamic lung compliance prone and supine positions newborn infant

Table 2 *Information from the questionnaire in relation to actual findings of motor disturbances*

Questionnaire	Actual findings of motor disturbances (Percent of children)				
	Minimal brain dysfunction (n=57)	Other neurological disorders (n=9)	Already under care for neuro-motor disorders (n=13)	Without neurological disturbances (n=2 373)	Total (n=2 447)
Boys	82.7	66.7	46.2	51.3	52.0
Girls	17.5	33.3	53.8	48.7	48.0
Socio-economic group I	21.[illegible]	44.4	30.8	32.4	32.1
II	28.8	44.4	30.8	34.5	34.4
III	50.0	11.1	38.5	33.2	33.3
Complications during pregnancy, partus or neonatal period	31.8	71.4	77.8	[illegible]5.4	25.9
Sitting without support first after 10 months	2.0	22.2	30.8	0.9	1.2
Walking without support first after 15 months	10.4	55.6	66.7	7.8	8.3
Speaking single words first after 15 months	22.4	22.2	41.7	12.8	13.3
Speaking sentences first after 2 ½ years	25.0	11.1	20.0	5.2	5.7
Marked difficulties in rearing	35.4	0	15.4	9.7	10.2
Hyperactive	25.5	0	0	11.4	11.6
Quarrelsome	10.2	0	0	3.2	3.4

Neurological findings. Most of the children 67 (2.7 %) were referred because of suspected neurological deviations such as clumsiness and failure to perform adequately the motor tasks presented during the medical examination (Table 1). Among these 52 children (43 boys and 9 girls) were classified as having *minimal brain dysfunction, MBD*. This was based on the findings of general clumsiness and fine *muscle incoordination in combination with* excessive mobility and further on restlessness and short attention span during the different items of the health control: testing of vision and hearing, medical examinations and psychological examination (33, 38, 39, 54).

In 27 of them no other neurological signs were found besides the clumsiness and hyperactivity. An EEG taken in 21 of them was normal. The remaining 25 children had other neurological disturbances as well. Six had mild ataxia or mild spasticity, 2 had a spontaneously arrested slight hydrocephalus. A pathological EEG was found as the *only other* neurological sign in 9 children: focal spike wave activity in ? girls, who were treated with anticonvulsants, and a diffuse slow activity in 6 boys and 1 girl, in 1 case thought to be sequelae after encephalitis. A retarded psycho-motor development was found in 8 boys; they were further investigated in cooperation with the Department of Child Psychiatry. One case of hydroxyprolinaemia was detected among them. The children with MBD received psychological, educational and social guidance, and some of them also pharmacological treatment and physiotherapy.

The remaining 15 children referred for neuro-muscular deviations constituted a mixed group. One girl had a slight hemiplegia detected in infancy but not adequately treated. One boy had a hereditary polyneuropathia type Charcot-Marie-Tooth and 3 children had a benign familial myopathia. Physiotherapy was instituted in these children.

A positive Babinski reflex, unilateral in 2 cases and bilateral in 2 cases, was the indication for referring 4 of the children. The findings were verified, but no other neurological abnormalities were demonstrated.

Suspicion of nocturnal psychomotor seizures caused the referral of 3 *children*. The clinical and electroencephalographic examinations

Table 1 *Clinical diagnosis of 76 children examined at the Department of Paediatrics*

	Boys	Girls	Total
Minimal brain dysfunction (clumsiness and hyperactivity)	*43*	*9*	*52*
A Without additional neurological signs	22	5	27
B Combined with mild ataxia or spasticity	5	1	6
C Combined with pathological EEG	6	3	9
D Combined with retarded psycho motor development	8	0	8
E Combined with a spontaneously arrested mild hydrocephalus	2	0	2
Other neurological deviations	*5*	*4*	*9*
F Slight hemiplegia	0	1	1
G Hereditary polyneuro pathia	1	0	1
H Benign myopathia	2	1	3
I Pos Babinski reflex without other signs of neurological disturbances	2	2	4
Other findings	*8*	*7*	*15*
Pavor nocturnus	2	1	3
Gait disturbance	0	1	1
Normal	1	1	2
Hepatomegalia	1	0	1
Chronic obstipation	0	1	1
Malabsorption?	1	0	1
Asthma bronchiale	1	0	1
Chromosome aberration?	1	0	1
Physiological cardiac murmurs	1	2	3
Post infectious myocarditis	0	1	1
Total number of children	*56*	*20*	*76*

2 *General physical examination*

By a careful inspection and palpation of the undressed child abnormalities of the mouth throat superficial lymph nodes thyroid gland skin skeleton muscles and abdomen were recorded In boys the testes and the foreskin were examined Heart and lungs were auscultated pulsations of the femoral arteries were palpated and at the end of the examination the auscultatory blood pressure of the left arm was measured using a 9 cm wide cuff on th supine child (31)

3 *Neuromuscular examinations*

Posture and spontaneous motor behaviour were ob served with the child sitting standing walking and running Attention was paid to asymmetry in stature and movements and also to the presence of involuntary movements The active power and the resistance to passive movements in head hands arms feet and legs were noted The knee ankle biceps and triceps jerks as well as the plantar reflex were elicited

Further assessment of the gross movements and their coordination was made while the child was walking along a straight line walking on tipto and on the heels and standing or hopping on one leg Th small movements and their coordination were studied while the child was pouring water from a can into a cup was cutting a piece of pap r and was threading small wooden beads on a string

Inappropriate activity and disordered attention span in a child during the examination were also recorded e g constantly moving around touching and handlin objects to no discernible purpose

4 *Haemoglobin concentration*

Determination of haemoglobin concentration was made from capillary blood samples and measured spectrophotometrically as haemoglobin cyanide as proposed by the European Society for Haematology (20)

Methods used in this physical examination are well known among paediatricians and partly included in recommended standards of comprehensive health care of children (7 8 11 49 51)

At the Child Health Centres all findings were discuss d with the accompanying parent and advice and treatment for minor problems were offered

Children with health problems considered to be functionally important at the present or in the future and not under current professional care were referred for further evaluation to the respective departments of the University Hospital of Lund

Statistical methods

In the statistical treatment Chi square analysis in case of four fold tables with Yates correction and t sts for normal distribution were used Computations were performed at the Computer Centre of Lund University (Univac 1108)

RESULTS

Altogether 164 children (125 boys and 39 girls) or 6 7 were referred for examination by various specialists subsequently 144 of them were examined and reported back

Paediatric findings

For further paediatric evaluation 81 children (58 boys and 23 girls) 3 3 were referred Five children failed to appear male twins with hereditary hypercholesterolaemia detected in infancy but not controlled since then and 3 girls with minor motor disabilities The clinical diagnosis of the remaining 76 children are summarized in Table 1

Table 5. Weight, height and head circumference in 1815 children aged 4 years ± 3 months in relation to area of residence, sex and socio-economic group

	n	Weight kg Mean	Weight kg S.D.	Height cm Mean	Height cm S.D.	Head circumference cm Mean	Head circumference cm S.D.
Lund boys							
Socio-economic group							
I	299	17.4	1.6	104.8	3.5	1.8	3.4
II	295	17.4	2.3	104.8	7.3	51.5	3.3
III	279	17.2	2.8	103.9	9.7	51.1	4.6
Sum	873	17.3	2.3	104.5	7.2	51.5	3.8
Lund girls							
Socio-economic group							
I	84	16.9	2.9	102.9	13.0	50.6	3.3
II	274	17.4	2.1	104.7	[illegible]	50.5	3.3
III	233	17.0	2.3	103.7	4.3	50.2	3.4
Sum	791	17.1	2.3	103.8	8.5	50.4	2.9
Dalby boys							
Socio-economic group							
I	3	18.0	1.9	104.3	1.2	52.0	0.5
II	35	17.4	2.0	104.9	4.8	51.6	2.2
III	37	17.1	2.3	104.7	4.0	49.9	8.5
Sum	75	17.3	2.1	104.8	4.2	50.8	6.0
Dalby girls							
Socio-economic group							
I	8	17	1.7	104.0	4.6	50.9	1.5
II	26	17.1	1.9	103.8	3.7	50.9	1.3
III	42	17.4	2.8	104.3	4.7	50.6	1.4
Sum	76	17.3	2.4	104.1	4.3	50.7	1.3
Boys total	948	17.3	2.3	104.6	7.0	51.4	4.0
Girls total	867	17.1	2.3	103.8	8.2	50.5	[illegible]
Grand total	1815	17.2	2.4	104.2	7.6	51.0	3.3

retentio testis, both ectopic and true undescended, were operated, as well as the hypospadias and the cervical fistula. The treatment of a boy with protruding ears was postponed until the age of 6–7 years. The girl with clitoris hypertrophy had no signs of hormonal hyperactivity; the hypertrophy was removed at the age of 7 years because of mechanical voiding difficulties.

Orthopaedic findings. Forty three children (2.4%), 30 boys and 13 girls were referred to

Table 6. Systolic and diastolic blood pressure in 1159 children aged 4 years ± 3 months

	n Boys	n Girls	Mean Boys	Mean Girls	S.D. Boys	S.D. Girls
Systolic	594	565	100	100	7.7	7.8
Diastolic	594	565	54	54	6.8	7.2

the orthopaedic surgeon. Nine children, 3 boys and 6 girls, all with flat feet, failed to appear. The diagnoses of the remaining 34 children are shown in Table 4. Two children walked with inwardly rotated feet, which very much worried their mothers, who both happened to be physiotherapists. The professional examination, however, revealed nothing abnormal. One girl had been treated for congenital luxation of the hip when she was newborn, and the parents had been advised to check the girl at the age of 4–5 years. She was clinically and roentgenologically normal. The remaining 31 children had pronounced flat feet combined with complaints from the feet or the legs. They were all treated with metal foot supports in their shoes (type Lange).

Anthropometrical measurements

Among the participating children, 948 boys and 867 girls, altogether 1 815, were examined at the age of 4 years ± 3 months. The weight, height and head circumference of these children according to area of residence, sex and socio-economic grouping are given in Table 5. There was no significant difference in mean height and weight between boys and girls or between children in Lund and Dalby ($p > 0.05$). In Lund, girls in socio-economic group II were somewhat taller and heavier ($p < 0.05$) than girls in group I and III. In Lund, boys had a larger head circumference than girls ($p < 0.001$). It may also be noted that children with reported feeding problems were shorter and lighter than children without such problems: mean height 103.2 and 104.1 ($p < 0.05$) and mean weight 16.9 and 17.5 ($p < 0.001$).

Table 3 *Clinical diagnosis of 34 children referred for surgical treatment*

Clinical diagnosis	Number of children Boys	Girls	Total
Retentio testis	8	—	8
Phimosis	12	—	12
Hypospadia	1	—	1
Hernia inguinalis	6	2	8
Clitoris hypertrophy	—	1	1
Infected wound	1	0	1
Medial cervical fistula	1	0	1
Cheiloschisis	1	0	1
Protruding ears	1	0	1
Total no of children	31	3	34

were normal however and the diagnosis was pavor nocturnus

In 2 of the referred children the neurological examination was normal One girl however had received spectacles because of hyperopia between the two examinations

A girl with gait disturbances was found to have a short leg and was treated with an extra heel on one shoe

Information from the questionnaires In Table 2 some information from the parents regarding the children and their development and behaviour is compared to the actual findings of motor disturbances

Children with MBD compared with normal children were significantly more often boys than girls ($p<0\ 001$) and slightly more often belonged to lower socio economic groups ($p<0\ 05$) they were more often regarded as difficult to handle ($p<0\ 001$) hyperactive ($p<0\ 01$) and quarrelsome ($p<0\ 05$) they talked late ($p<0\ 001$) but did not sit or walk late more frequently than normal children ($p>0\ 05$)

Among the children with neurological disturbances (52 MBD 9 other neurological disorders and 13 already under professional care total 74 children) there were significantly more children with late motor and speech development than among healthy children ($p<0\ 001$) Also complications during pregnancy (toxaemia diabetes) parturition and neonatal period (prematurity strong icterus or cyanosis convulsions breathing difficulties) appeared more frequently in these children ($p<0\ 001$)

Cardiological findings Four children were referred 3 because of rough systolic murmurs and 1 because of cardiac arrhythmia The cardiac murmurs were all considered to be physiological and the girl with arrhythmia was found to have a post infectious myocarditis which showed regression at subsequent follow ups

Other findings Among the children with non neurological deviations (Table 1) 1 boy had a palpable liver Clinical and laboratory investigations were normal and an X ray examination revealed a liver of normal size but with a depression of the right lobe considered to be a normal variation

As a newborn 1 boy with multiple malformations was suspected of having a chromosome aberration Now at the age of 4 years a new analysis showed normal karyotype

Surgical findings Forty children (1 6) 37 boys and 3 girls were referred for surgical treatment Six boys 5 with phimosis and 1 with suspected urachus fistula did not keep their appointment with the paediatric surgeon The diagnoses of the remaining 34 children are summarized in Table 3 Phimosis, in combination with recurrent balanitis or voiding difficulties was the most frequent cause for referral (12 boys) Seven of them were treated surgically with circumcision or lysis under general anaesthesia in the remaining 5 boys a simple dilatation and retraction of the fore skin was performed All inguinal hernia and

Table 4 *Clinical diagnosis of 34 children referred to the orthopaedic surgeon*

Clinical diagnosis	Number of children Boys	Girls	Total
Flat feet	26	5	31
Control of congenital luxation of hip	0	1	1
Rotation of feet	1	1	2
Total no of children	27	7	34

Table 5. *Weight, height and head circumference in 1815 children aged 4 years ± 3 months in relation to area of residence, sex and socio-economic group*

	n	Weight kg		Height cm		Head circumference cm	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
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Socio-economic group							
I	3	18.0	1.9	104.3	1.2	52.0	0.5
II	35	17.4	2.0	104.9	4.6	51.6	1.1
III	37	17.1	2.3	104.7	4.0	49.9	8.5
Sum	75	17.3	2.1	104.8	4.2	50.8	6.0
Dalby girls							
Socio-economic group							
I	8	17.2	1.7	104.0	4.6	50.9	1.5
II	26	17.1	1.9	103.8	3.7	50.9	1.3
III	42	17.4	2.8	104.3	4.7	50.6	1.4
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Table 7. *Haemoglobin concentration (g/100 ml) in 2 218 4-year-old children in relation to area of residence, sex and socio-economic group*

	n	Mean	S.D.
Lund boys			
Socio-economic group			
I	362	12.65	0.66
II	351	12.69	0.69
III	329	12.66	0.79
Total	1 042	12.67	0.71
Lund girls			
Socio-economic group			
I	327	12.76	0.72
II	323	12.75	0.69
III	285	12.67	0.73
Total	935	12.73	0.71
Dalby boys			
Socio-economic group			
I	8	12.55	0.71
II	50	12.37	0.73
III	61	12.28	0.70
Total	119	12.34	0.71
Dalby girls			
Socio-economic group			
I	16	12.38	0.80
II	44	12.54	0.66
III	62	12.41	0.77
Total	122	12.45	0.73
Boys, total	1 161	12.63	0.72
Girls, total	1 057	12.70	0.72
Grand total	2 218	12.67	0.72

Blood pressure

Blood pressure recordings were made in 1 159 children, 594 boys and 565 girls. As shown in Table 6, the mean blood pressure was the same for boys and girls, 100/54.

In a few children the initial values were elevated but were normalized on further readings. Persistently elevated diastolic blood pressure above 2 S.D. (23) was not found in any case.

Haemoglobin concentration

Haemoglobin measurements was performed on 2 218 children. The results in relation to area, sex and socio-economic grouping are given in Table 7. Both boys and girls had a significant higher mean Hb in Lund than in Dalby ($p < 0.001$). In Lund, girls had a somewhat higher mean Hb than boys ($p < 0.05$). This sex difference was not significant in Dalby ($p > 0.05$). No difference in mean Hb was found between the socio-economic groups either in Lund or in Dalby.

In total, 54 children (2.4%) had a haemoglobin concentration at or below the mean −2 S.D. (11.2 g/100 ml). 1 had 9.8 g/100 ml, 4 had between 10.0 and 10.4 and 15 had between 10.5 and 10.9 g/100 ml. Anaemia, defined as haemoglobin concentration below 11 g/100 ml (35), was thus found in 20 children, 0.9%.

The cases of anaemia were equally distributed among boys and girls, in Lund and in Dalby and in different socio-economic groups ($p > 0.05$) (Table 8).

Children with reported feeding problems or frequent upper respiratory infections did not have anaemia more frequently than others ($p > 0.05$). In fact, there was no significant difference in mean Hb concentration between children who according to their mother's opinion had sufficient consumption of food and children who consumed disturbingly little or refused to eat for long periods (Hb 12.68 ± 0.71 vs. 12.69 ± 0.75).

Table 8. *Parental information about children with and without anaemia (Hb < 11.0 g/100 ml)*

	With anaemia (n = 20)		Without anaemia (n = 2198)		Total n = [illegible]18	
	n	%	n	%	n	%
Living in Lund	15	75.0	1962	89.3	1977	89.1
Living in Dalby	5	25.0	236	10.7	241	10.9
Boys	13	65.0	1148	52.2	1161	52.3
Girls	7	35.0	1050	47.8	1057	47.7
Socio-economic group						
I	3	15.0	710	32.3	713	3[illegible].2
II	7	35.0	761	34.6	768	34.6
III	10	50.0	727	33.1	737	33.[illegible]
Reported frequent upper respiratory infections (1 per month or more)	1	5.0	[illegible]41	11.0	[illegible]4[illegible]	10.9
Reported feeding problems	2	10.0	82	3.7	84	3.8

Table 9 *Clinical diagnosis of 41 children reported to be under current paediatric care*

	Boys	Girls	Total
A Convulsions	3	0	3
B Down's syndrome	2	0	2
C Myelomeningocele operated	0	1	1
D Hydrocephalus	1	1	2
E Cerebral palsy	0	4	4
F Werdnig Hoffman	0	1	1
G Hemicrania	0	1	1
H v Willebrand's disease	1	0	1
I Cyclic neutropenia	1	0	1
K Adipositas	0	3	3
L Verified or suspected organic heart disease	8	6	14
M Asthma bronchiale	4	3	7
N Ichthyosis	0	1	1
Total number of children	20	21	41

Current professional care

In the questionnaires 202 children were reported to be under current professional care 41 children by pediatricians 24 children by pediatric surgeons and 137 children by orthopaedic surgeons Their diagnoses were checked in the records and are presented in Tables 9–11 These children underwent the health examination but were not considered to be in need of further evaluation and were not referred

From the records at the Department of Paediatrics it was found that another 10 children among the 126 who failed to participate in the health examination were under current paediatric care 4 because of cerebral palsy 2 for convulsions 1 for each diabetes multiple malformations pancytopenia and organic heart disease

DISCUSSION

In a country where practically all children are born in hospitals with obstetric and paediatric services and where the vast majority of all infants are regularly followed by physicians at Child Health Centres it is quite obvious that gross physical disabilities and handicaps should be detected at an early age

The rationale for a thorough physical examination at the age of 4 years was the assumption that some minor disabilities although of some importance for the child might have been overlooked and others might have developed after infancy

This hypothesis was proved correct Physically the four year-old children were generally in good health Their *height and weight* were greater than those obtaining in Sweden some 30 years ago (1) and about the same as recently found by Samuelson in Northern Sweden (45) The reasons for the difference in height and weight between children with and without feeding problems could be that small children eat less or conversely that children who eat less become smaller In our affluent society the first assumption is the more likely

The *haemoglobin concentration* was generally satisfying and similar to that found in other comparable Scandinavian investigations (34 36) Also in accordance with these studies but contrary to results from USA (36 37) a very low frequency of anaemia was found It

Table 10 *Clinical diagnosis of 24 children reported to be under current surgical care*

	Boys	Girls	Total
Hypospadia	6	0	6
Phimosis	3	0	3
Retentio testis	4	0	4
Cheilognatopalatoschisis	2	4	6
Hernia inguinales	0	2	2
Hydronephrosis	0	2	2
Torticollis	1	0	1
Total number of children	18	6	24

Table 11 *Clinical diagnosis of 137 children reported to be under current orthopaedic care*

	Boys	Girls	Total
Pes plano-valgus and or genu valga	86	41	127
Club-feet	3	0	3
Malformation of the hip	3	0	3
Malformation of the spine	0	1	1
Pseudarthosis	1	0	1
Osteomyelitis	1	0	1
Damage of brachial plexus	0	1	1
Total number of children	94	43	137

might be supposed however, that children be low *mean* −2 *S D would* benefit from iron supplementation *(44)*

Available information does *not* explain the *difference in* mean *Hb* between Lund and Dalby and between girls and boys in Lund An investigation of nutrition and food habits might have provided the answer but this *type of* study was not included However no difference in *Hb* concentration was found between children with feeding problems and children with satisfactory food consumption as based on the parents opinions Of course children with feeding problems might have received supplementation with iron and vitamins to a greater extent than other children but we have no information on this

All severe *neurological disabilities* were already detected and under treatment (A–F *Table 9) However* another 61 children (2 5 %) were *found to have* neuro muscular disturbances Although the neuro paediatric examination revealed only minor neurological signs in most of these children it is evident that they did benefit from the identification Usually this led to measures such as pharmacological treatment physiotherapy and environmental changes including certain pedagogic techniques and parental counselling to avoid emotional and education trouble (4 9 *25 41 53)*

An extensor plantar response is known to occur as a sign of minimal brain damage (38) but as our 4 children with this phenomenon had no other abnormal neurological signs whatsoever and seemed to display normal activity and organization the findings were considered to be of no clinical importance (51)

It is impossible to predict how many of these motor disturbances will remain and appear as handicap later in life It seems reasonable however to assume that children with minimal brain dysfunction, hemiplegia and polyneuropathia may have difficulties in their further adjustment and function (15 38 39 41 54)

In this survey of the total population of 4 year-old children in a restricted area, 19 children (0 7 %) were already under current treatment for conditions affecting the neuro muscular system By including children with newly discovered motor disturbances of some significance (54 children 2 2 %) the figure of about 3 % with motor dysfunction is reached As expected boys predominate significantly

Paine (38) maintains that minimal brain dysfunction is the most common neurological diagnosis among children affecting 5 % or more or the entire random child population Our finding of 2 1 % must be regarded as a minimum figure It is quite conceivable that the number of children in this heterogeneous group will increase when greater demands for perception conceptualization language, memory and control of attention impulse or motor function are made upon the children in school

It must be emphasized again that MBD is a diagnostic label given to a very heterogeneous group of children who may or may not have neurological deviations However they have a disorder of function in common and this may well be developed solely by inadequate upbringing and certainly will be aggravated in such an environment (9 41) Furthermore families with a lower socio economic standard may have greater difficulties in handling over active children and this could explain our findings in this respect

Already it is evident that these children are a very disturbing element to their families, and it is important that every effort is made to reduce further exogenous harm to the children and to diminish the burden on the families

Comparison with results from other investigations is difficult as materials, methods and definitions show great disparity Usually information is collected retrospectively from medical and social records and mild disturbances are not registered According to our knowledge a complete study of an unselected

population of pre school children in one area has not previously been reported

von Sydow (50) reports motor handicaps in 5 32‰ of life births in a study of children 0–16 years in a Swedish county In two thirds of all cases the handicap was considered to be slight or negligible Information was gathered by questionnaires and records In an another Swedish county Lagergren (26) using the same method found 2 6‰ of children 4–16 years of age suffering an important motor handicap In a sample from the British cohort born during the first week of March 1946 Pless & Douglas (40) found on scrutinizing available documents a prevalence of chronic neurological disorders of 1 52

The *cardiac findings* in our study were sparse 4 children were referred and only one of them was found to have an affection of any importance a post infectious myocarditis (Table 1) The explanation must be that serious cardiac affections were detected at a younger age and were already under care (Table 9) and that innocent heart murmurs were diagnosed as such by the paediatrician at the Child Health Centre and not referred for further evaluation According to Carlgren (2) the frequency of congenital heart disease in Swedish live newborns is 7 7‰ and after 7–16 years about 4 in 1 000 children are still alive and have symptoms and signs of the disease These figures as well as those from screening examinations with a computer 3 1 per 1 000 pre-school children (42) and 4 2 per 1 000 school-children (5) are well covered in our study by the number of children already under surveillance by our paediatric cardiologist 15 among the total material of 2 573 4-year-old children or 6 per 1 000

The prevalence of *pulmonary findings* (asthma bronchiale) was low at this age Only 1 child was referred and another 7 children were reported to be under professional care because of asthma This gives a prevalence of 0 3% similar to that found by Collins in USA (3) Only 1 child in our study had frequent attacks and was disabled by his diseases Of course considerably more children have or have had attacks of infectious obstructive bronchitis and some may later be diagnosed as asthma (28)

According to several authors (13 30 31) routine measurements of the *blood pressure* should be performed on all patients regardless of age as it is becoming increasingly clear that essential hypertension may start at an early age (13 32 55) In our rather large material of healthy 4 year-old children however we failed to detect any case of hypertension The mean systolic and diastolic pressure is about the same as that found by Londe (31) in a smaller material

The most urgent *surgical findings* were the 8 boys with retentio testis Surgical treatment of these boys is recommended before the age of 5 years as the development of the undescended testis is retarded after that age and may lead to impaired fertility (27) Also it is probable that early orchidopexy gives some protection against malignancy (27) Together with the 4 boys already waiting for an operation (Table 9) the prevalence of retentio testis in this material was 0 9% (12/1 272) This is in close agreement with Scorer's statement that the true incidence of undescended testis in boyhood is probably 0 8 / (48)

The treatment of phimosis is a disputed subject It seems however that the retractability of the foreskin gradually increases as the tissues develop at least when no violent attempts are made to accelerate the normal course of nature (12 56) Therefore only boys with recurrent balanitis or mechanical voiding problems were referred for surgical evaluation The mothers of all other boys with a tight prepuce or preputial adhesions were advised to await a spontaneous widening and separation

Also with regard to *orthopaedic findings* the more obvious and important deviations such as congenital malformations were detected and treated long before the age of 4 years Only minor disabilities with little influence on the child's development and well

might be supposed, however that children below mean −2 S D would benefit from iron supplementation (44)

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being were diagnosed in our study In other surveys of pre school children the frequencies of orthopaedic problems vary a great deal again depending on sampling methods and criteria Sometimes especially in German investigations unrealistically high figures are presented

Thus orthopaedic conditions were found in 1 4° of 20 000 children 0–6 years of age in New York City (18) in 3 4 of 959 children 0–5 years of age in Minneapolis (52) and in 15 2° of pre-school children in East Germany (47) Among 1 332 4-year old children in Kiel W Germany 65 5% had orthopaedic deviations and 27° were considered to be in need of treatment (19) Foot deformities was detected in 60% of 4 064 4–6 year-old children in Sinsheim W Germany (16)

In this study too orthopaedic and foot problems especially seemed to be of some concern to doctors and parents as 5 2 of our children were under current care for flat feet Practically all were prescribed with metal foot supports although few were actually using them Since most of the minor postural problems of the feet and legs of the small child seem to correct themselves during normal growth and activity of childhood (10 14 29 43) it may be wise to restrict the orthopaedic referrals to children with major deformities or apparent complaints from their extremities

Although much valuable information about the child and his family was gathered in the *questionnaire* it is evident that a parental evaluation in the present form can hardly serve as a screening instrument to detect neurological aberrations in the children Whether it is necessary to offer a complete professional examination at a certain pre school age to reveal physical health problems is a matter of medical and economical opinion and will be discussed at some length in a later paper

SUMMARY

An unselected population of 2 447 four year old children in two communities in southern Sweden underwent a physical examination as part of a general health control The general health was very good mean weight was 17 2 kg mean height 104 2 cm mean Hb 12 7 g/100 ml Anaemia (<11 g/100 ml) was detected in 20 children (0 9%) The mean blood pressure was 100/54 No child with persistently elevated blood pressure was found

Practically all serious handicaps were detected and already taken care of before the age of 4 years

Altogether 164 children (6 7%) were referred to specialists for newly detected deviations, and 144 (5 9) were reported back In 52 children 2 2° minimal brain dysfunction was diagnosed, half of them having other neurological disturbances besides clumsiness and hyperactivity Five children (0 2%) had other neuro muscular deviations such as myopathia hemiplegia polyneuropathia Including children already under professional care 3% showed a significant degree of motor dysfunction

The cardiac findings were sparse the prevalence of heart diseases at this age was about 6/1 000 all already detected

Phimosis was the cause of referral in 12 boys (0 9) The prevalence of retentio testis was 0 9 at this age

The orthopaedic findings were also confined to minor disabilities with little influence on the child s development and well being 31 children (1 3) had pronounced flat feet with complaints from the feet or the legs Another 5 2 were already under current care for flat feet

From a questionnaire to the parents, some children could be designated as having neurological disorders but the information was not selective enough to be of practical value as a screening instrument

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VECTORCARDIOGRAPHIC EXAMINATIONS IN DEXTROCARDIAC CHILDREN

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The ECG changes of patients with positional anomalies have already been studied by several authors (2 3 11 12 15) Examination by ECG and vectorcardiography (VCG) facilitates the localization of the atria atrial overload and rhythm disorders The sequence of activation provides useful information for localization of the ventricles Especially in transposition complexes the ECG is of limited value in detecting ventricular hypertrophy The purpose of the present paper is to study the method of choice of VCG examination type and its value in cases with dextrocardia

MATERIAL AND METHOD

Fifteen cases with dextrocardia were studied The classification and distribution of the different forms was as follows

1 *Isolated dextrocardia*, without visceral inversion 9 cases Children with dextroversion or dextrorotation with or without heart disease were placed in this group

2 *Situs inversus totalis* with visceral inversion and dextrocardia 4 cases Mirror image dextrocardia is also classified under this heading the term being used only for children without heart disease

3 *Dextrocardia with indetermined visceral asymmetry* liver mesenterium commune etc usually forming part of asplenia or polysplenia syndrome cases.

Children with secondary dextroposition of extracardiac origin without heart disease were excluded from this material

Using the Frank system (5) in supine position the VCGs were registered in three planes (horizontal right sagittal frontal) with Hewlett Packard 1507/a vectocardiograph

In dextrocardiac patients, there are certain recording differences The abnormal position of the heart in relation to the recording electrodes causes changes the magnitudes of which are related to the electric distances These changes are more frequent in the *x* axis and fewer in the *z* axis resulting in distortions of the VCG loops If the VCG electrodes are applied by the usual system the presuppositions on which resistance circuits were calculated by Frank for his experiments on a human torso model are absent

With the aim of evaluating the importance of the electrode placements in different types of dextrocardia VCGs were recorded not only in the usual way according to the Frank method but also in inverted electrode position—the electrodes of the A and I points were interchanged and electrode C was placed on the right side in a position exactly corresponding to its usual left side position.

In cases with situs inversus totalis the inverted electrode position more accurately reflects the spatial electromotive forces The inverted polarity of the *x* axis however gives some misleading results when compared with the ECG patterns which are recorded in the usual way and with ordinary polarity

In Fig 1 *a* the picture of a child with typical situs inversus totalis mirror image dextrocardia, and no detectable heart disease is presented As the ordinary type of Frank recordings clearly show the form of the VCG loop in the horizontal plane is not entirely the mirror image picture of the usual normal one and does not correspond completely to the ECG picture Placing the VCG electrodes in the inverted position already mentioned the inscriptions will be normal (Fig 1 *b*) These records do not correspond to the actual cardiac situs as is best seen when the records from inverted electrodes and the inverted polarity of the *x* axis are compared with the ECG they are in complete dysharmony Finally the real patterns which correspond to the heart situs and ECG are those which result from the mirror image picture of the VCG in the inverted position (Fig 1 *c*) If no quantitative estimation is required this last procedure can be omitted simply because the VCG loops for normally positioned hearts (see Fig 1 *b*) are easily

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In Fig 1 *a* the picture of a child with typical situs inversus *totalis mirror image dextrocardia and no* detectable heart disease is presented As the ordinary type of Frank recordings clearly show the form of *the VCG loop in the horizontal plane is not entirely* the mirror image picture of the usual normal one and does not correspond completely to the ECG picture *Placing* the VCG electrodes in the inverted position already mentioned the inscriptions will be normal (Fig 1 *b*) These records do not correspond to the actual cardiac situs as is best seen when the records from inverted electrodes and the inverted polarity of the *x* axis are compared with the ECG they are in complete dysharmony Finally the real patterns which correspond to the heart situs and ECG are those which result from the mirror image picture of the VCG in the inverted position (Fig 1 *c*) If no quantitative estimation is required this last procedure can be omitted simply because the VCG loops for normally positioned hearts (see Fig 1 *b*) are easily

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Table 1 *The distribution of cases according to the cardiac and visceral positions complicating anomalies the basis of diagnosis and the spatial half area QRS T angles*

No	Pt	Age	Sex	Cardiac position	Complicating anomalies	Heartcath and angiogr	Sp h a QRS–T angle
1	H J	2 y	M	Isol D	—	–	26 (31)
2	L J	11 y	M	Isol D	Defect costae III–IV	+	31 (27)
3	L M	5 y	F	Isol D	—	–	10 (19)
4	V E	12 y	M	Isol D	—	+	17
5	C R	8 y	M	Isol D	—	–	20 (32)
6	A J	16 y	M	Isol D	—	–	18 (19)
7	R L	8 y	M	Isol D	—		13 (35)
8	D J	9 y	M	Isol D	ASD omphalocele	+	38 (45)
9	P P	11 y	F	Isol D	VSD omphalocele	+	44
10	J V	12 y	M	SI tot	—	–	10 (8)
11	Y M	1 y	F	SI tot	VSD PS corr TGA	+	77
12	R K	5 m	F	SI tot	VSD PS TGA	+	135
13	K J	3 y	F	D indet visc sit	PS TGA single ventr asplenia	+	152
14	E O	15 y	M	SI tot	VSD PS corr TGA	+	55 (98)
15	K S	15 y	F	D indet visc sit	PS TGA single ventr	+	150 (142)

In 3 children with inverted atrial situs (cases 10 14 15) we examined the VCG recorded in both the ordinary and inverted electrode position

The VCG inscriptions were examined quantitatively measuring vector angles in degrees and magnitudes in mV QRS instantaneous vectors of 10 20 30 msec maximum and half area vectors were calculated Terminal forces were estimated by the Abbott Smith & Chou method (1) because terminal timed vector measurements seemed to be inaccurate In addition the magnitude and angle of T maximum vector were examined All measurements have been taken in the horizontal and frontal planes The spatial magnitude of 10 20 30 msec instantaneous vectors the maximum QRS half area QRS and terminal vectors were calculated according to the Pythagorean theorem using Withams method (17) For spatial half area QRS-T angle measurements Brinberg s method modified by Hannonen was used (8)

RESULTS

In cases with isolated dextrocardia with normal atrial situs and concordant ventricles (i e in dextroversion) without any heart disease (cases 1 7) the anterior and rightward displacement of the QRS loops were present in the horizontal plane It corresponded to the typical dextroversion ECG

In case 8 the dextroversion was complicated by an atrial-septal defect with a small left to right shunt The VCG showed signs of dextroversion complicated by patterns of incomplete right bundle branch block

In case 9 besides dextroversion ventricular septal defect was present with a small left to right shunt The pressure in the right ventricle was normal The VCG showed only signs of dextroversion

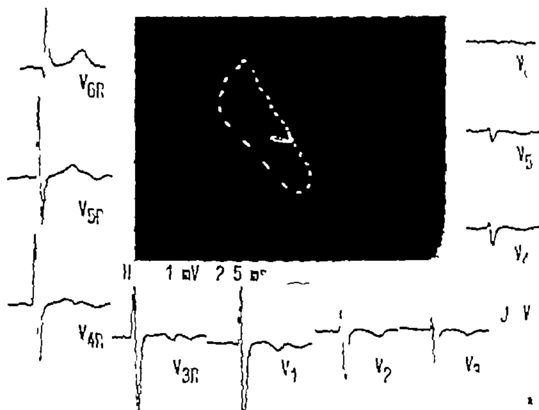
In case 10 in mirror image dextrocardia the VCG patterns (Fig 1c) corresponded to the mirror image of normal ones

In our material the number of cases with inverted atrial situs and associated defects (all including the transposition complexes) was 5 In 3 cases VCGs were recorded with the ordinary electrode position and in 2 cases with the ordinary and inverted electrode position As an example the horizontal plane pictures of case 14 were presented (Fig 2 a–c)

In every case examined the VCG in sagittal plane showed unimportant differences between the ordinary and inverted electrode placement recordings (cases 1–8 10 11 14 15)

DISCUSSION

Only four articles have been found dealing with VCG changes in different forms of dextrocardia (2 3 10 16) but only two of them pay attention to the special electrode applications Sangiorgi et al (16) used Jouve s system (9) Miller et al (10) Grishman s cube system (7)





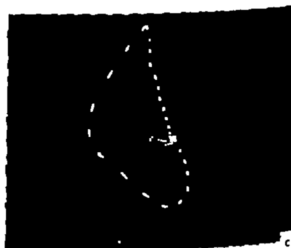


Fig 1 ECG and VCG records of case 10 with mirror image dextrocardia (*a*) with ordinary Frank electrode placement in horizontal plane (*b*) with inverted electrode placement (*c*) with inverted electrode placement presented in mirror image picture

recognizable consequently distortions of the riptions caused by ventricular hypertrophy are more easily recognized It must however be kept in mind that with the inverted electrode position the polarity of the x axis is interchanged In this study for reasons of comparison the measurements were calculated from mirror image pictures

Table 1 shows the distribution of cases according to the cardiac and visceral positions complicating anomalies age and the basis of diagnosis

The cases with isolated dextrocardia without atrial and ventricular inversion were all examined using the ordinary Frank method and in cases 1 2 3 5 6 7 8 using both ordinary and inverted electrode position In this group the results with normal electrode placement proved to be more appropriate in relation to the ECG than with its inverted electrode position Therefore we have taken measurements from the VCG records using the ordinary Frank electrode placement in cases without atrial and ventricular inversion

Table 1 *The distribution of cases according to the cardiac and visceral positions complicating ano malies the basis of diagnosis and the spatial half area QRS T angles*

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In every case examined the VCG in sagittal plane showed unimportant differences between the ordinary and inverted electrode placement recordings (cases 1–8 10 11 14 15)

DISCUSSION

Only four articles have been found dealing with VCG changes in different forms of dextro cardia (2 3 10 16) but only two of them pay attention to the special electrode applications Sangiorgi et al (16) used Jouve s system (9) Miller et al (10) Grishman s cube system (7)

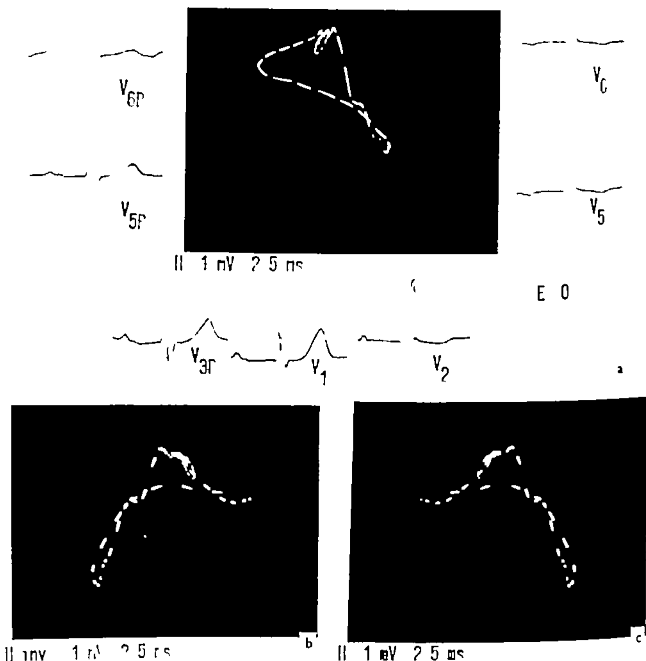


Fig 2 ECG and VCG patterns of case 14 with situs inversus totalis pulm sten VSD and corrected transposition of the great arteries (*a*) with ordinary Frank electrode placement in horizontal plane (*b*) with inverted electrode placement (*c*) with inverted electrode placement in mirror image picture

and inverted cube system Thus the results in this study are not really comparable Miller concluded that inverted cube electrode applications are necessary only in cases with atrial or ventricular inversion and not in cases with dextroversion

According to the results of this study in dextroversion inverted electrode placement is not necessary with the Frank system As for inversion and transposition complexes though the small number of cases dealt with here seem to suggest that the ordinary electrode placement in the Frank system does not result in considerable distortions as in Grishman s cube method further examinations are necessary

The examination of spatial QRS T angle is

a fundamental criterion in VCG interpretation as suggested by Pipberger et al (13 14) and Grant et al (6) The spatial half area QRS T angle was found by Hanninen (8) to form one criterion for distinguishing between pathologic and physiologic ventricular hypertrophy in the newborn period

The recognition of myocardial hypertrophy is of special importance in dextrocardia because the changed cardiac position itself without any associated anomaly can produce signs in the ECG which are otherwise typical for right ventricular hypertrophy The present authors have carried out vectorcardiographic examinations the results of which have not yet been published on patients with ventricular septal defect and patent ductus arteriosus and these examinations show that the spatial half area QRS-*T* angle can be regarded as a good indicator of myocardial hypertrophy

The spatial half area QRS *T* angle (Table 1) was measured in every patient with dextrocardia In cases without associated anomalies (cases 1-7 and 10) and in children with unimportant left to right shunts (case 8 and 9) this angle was small It is of interest that when the spatial half area QRS *T* angle in ordinary and in inverted electrode positions was calculated although it varied in size it was always less than 50 According to our above mentioned examinations and Hanninen (8) this angle can be regarded as normal

In transposition complexes (cases 11–15) this angle was in both ordinary and inverted electrode positions between 70 and 152 except in case 14 for whom it was 55 with usual electrode placement Such a large value can be regarded as an indication of ventricular hypertrophy

Thus the spatial half area QRS-*T* angle which is not influenced by heart position seems to be a valuable indication of myocardial hypertrophy

SUMMARY

Fifteen cases with several forms of dextrocardia were examined by vectorcardiography according to the Frank system In cases of isolated dextrocardia with concordant visceroatrial and atrioventricular situs without transposition heart disease or with small left to right shunt the results corresponded to the ECG The VCG differed only slightly from the mirror image of inscriptions with inverted electrode placement In situs inversus totalis with mirror image dextrocardia and in transposition complexes the usual VCG records were more distorted and did not correspond to the ECGs Further investigations are necessary with the inverted electrode placement The spatial half area QRS *T* angle appeared to be useful in estimating ventricular hypertrophy and was not influenced by the position of the heart

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SHORT COMMUNICATION

DECREASING INCIDENCE OF LOW BIRTH WEIGHT DIPLEGIA—AN ACHIEVEMENT OF MODERN NEONATAL CARE*

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Only fragmentary information exists concerning the effect of modern preventive and therapeutic measures in the delivery room and during the neonatal period on the incidence and distribution of cerebral palsy syndromes When recently examining the changing panorama of these syndromes in a broad field study in Sweden (3) preliminary data of some relevance to the care of premature babies became evident

CLINICAL MATERIAL

All children with cerebral palsy born in 1959-68 in the town of Gothenburg the county of Uppsala and the four counties belonging to the Western County Region were reevaluated and classified according to the system used in Sweden since 1958 (1 4) The material was considered to be unselected and complete Statistical calculations concerning the differences between the first and second five year periods were performed on each syndrome separately However for the purpose of this particular study spastic diplegia and ataxic diplegia were grouped together The approximate rate of deliveries in obstetric units in Sweden during the years in question was 99%

RESULTS

During the second five year period (1964-1968) a significantly decreased total incidence of cerebral palsy was revealed (Table I and Fig 1) This was found to be due to a likewise significantly lowered number of diplegic babies and among them of those who were prematurely born with a birth weight less than 2 500 g The rate of premature births was found not to have changed during the period studied Other striking findings were the absence in the whole period of any case of pure choreoathetosis due to kernicterus and the small number of more severe dyskinetic (dystonic tonus changing) infants However these changes in the panorama of cerebral palsy syndromes must evidently be referred to factors occurring before 1959

DISCUSSION

The syndrome of spastic diplegia is heterogeneous and can be divided into at least two main aetiological sub group as is strongly supported among other things by the bimodal birth weight distribution Prenatal factors seem to preponderate in full term infants (2 5) while neonatal factors would seem to be of major importance for infants of very low birth weight as evidenced by the studies of McDonald (7) The available data suggest that particularly the paraplegic form of spastic diplegia is a disease of the child who is born immature (8)

Experiences from clinical medicine and animal studies have revealed a surprisingly good resistance to pure oxygen deprivation in premature individuals When combined with derangement of homeostasis the situation may

Table 1 *Incidence of CP syndromes during the two five year periods 1959–1963 and 1964–1968*

		Inc per 1 000 live births 59-63	64-68	*p*
Spastic syndromes	Hemiplegia	0 61	0 55	
	Tetraplegia	0 06	0 04	
	Diplegia	0 79	0 55	0 01
Ataxic syndromes	Ataxic diplegia	(incl. above)		
	Cong ataxi	0 11	0 17	
Dyskinetic syndromes	Mainly athetot	0 08	0 05	
	Mainly dystonic	0 21	0 17	
Total no CP		1 86	1 54	0 05
Total diplegic syndromes	B w >2 500 g	0 31	0 26	
	B w ≤2 500 g	0 48	0 29	0 01

be quite different and there are many possible causes of selective intracellular damage and many metabolic abnormalities in premature infants (7)

The decreasing incidence of low birth weight diplegias from the middle of the sixties noted in the present study *coincides in time* with the introduction in Sweden of *new routine procedures* in the care of premature babies It is tempting to believe that the careful supervision and prevention of acidosis hypocaloric states and hypoglycaemia *ad modum* Usher (9) might have been of importance This opinion has recently been strongly supported by interesting experimental research on premature lambs by Kjellmer et al (6) These authors showed that experimentally induced asphyxia resulted in various signs of disturbed cerebral function at a significantly higher P_O level when acidosis was added

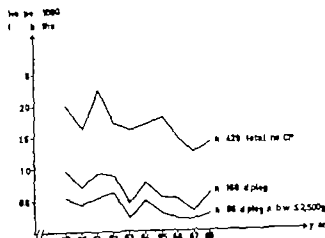


Fig 1 Incidence of diplegia through the years 1959–1968 compared to the total incidence of CP Total number of cases 429

SUMMARY

The clinical syndromes of 429 cerebral palsied children born in 1959–68 and constituting a representative and unselected Swedish series were analysed with respect to the changing panorama of the various syndromes The main preliminary finding was a significantly decreased total incidence due to a likewise significantly lowered number of diplegic babies with a birth weight less than 2 500 g

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CASE REPORT

THE OCCURRENCE OF TWO VASCULAR RINGS IN THE SAME INFANT

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Vascular rings and related malformations of the aortic arch are a rather common cause of stridor respiratory and feeding difficulties during infancy (1–3 5) Most frequently the vascular rings are the results of anomalous development of one or several of the primitive aortic arches But many cases caused by a left pulmonary artery with an anomalous origin and course have also been reported In these cases the malformation has been called vascular sling (4) It often occurs with concomitant congenital heart defects and malformation of the trachea

This report will draw attention to the possibility of the occurrence of a vascular ring and a vascular sling in the same patient

CASE REPORT

Boy born in a normal delivery with a birth weight of 4 430 g The pregnancy and familial history were uneventful

At the age of 2 months the infant was admitted to hospital because of vomiting and failure to thrive On admission his general condition was good He had no respiratory difficulties or signs of cardiac failure A holosystolic murmur was heard at the left sternal border with maximal intensity in the fourth intercostal space The electrocardiogram showed signs of right ventricular hypertrophy but no definite signs of hypertrophy of the left ventricle Roentgen examination demonstrated enlargement of the heart, especially the right atrium and ventricle and the left atrium The pulmonary vessels were wide as in left-to-right shunt Roentgen examination of the oesophagus was performed because of vomiting but no pathologic changes were found (the examination was however not performed with the patient in a true lateral position)

A few days after admission to the hospital congestive heart failure developed He was treated with digitalis and diuretics and improved

Two weeks later stridor and respiratory difficulties developed Atelectasis of the middle and right lower lobes were found at roentgenexamination Th trachea was very narrow the sagittal diameter was 2 3 mm (Fig 1) but there was no local indentation

In spite of treatment with antibiotics broncholytic drugs and hydrocortisone his condition deteriorated An endotracheal tube (French size 2 5) could not pass into the trachea and because of increasing respiratory difficulties a tracheostomy was performed At a new roentgen examination of the oesophagus a posterior impression was observed in th upp r part of the oesophagus Just below this there was an anterior impression and at the same level an increased distance between oesophagus and trachea

To outline the anatomy of the supposed vascular ring and the congenital heart disease a cardiac catheterization was performed The pressure in the right ventricle and pulmonary artery was found to be increased (systolic pressure about 60 mmHg) Oxygen saturation data revealed left to right shunt at ventricular level calculated ratio lung blood flow/systemic blood flow 2 1

Angiocardiography with contrast injection into the right ventricle was performed (Fig) The right sided atelectasis caused dislocation of the heart and mediastinum to the right The left pulmonary artery branched from the right pulmonary artery and made a hook around the right border of th trachea and passed between the trachea and oesophagus to the



Fig 1 Lateral projection of oesophagus and trachea The upper oesophagus is impressed from behind by the left subclavian artery The distance between the lower part of the trachea and oesophagus is increased Trachea (*arrows*) is narrow

left lung (this anomalous origin of the left pulmonary artery was not noted at the initial examination the peculiar loop of the pulmonary artery was thought to b due to the dislocation to the right of all cardiac structures) Both the ascending and descending aorta were situated to the right of the trachea The right brachio-cephalic arteries and the left carotid artery branched off from the ascending aorta Th left subclavian artery came from the descending aorta and crossed the midline behind the oesophagus Ductus arteriosus completing the vascular ring was not demonstrated but the anatomy of the aorta and the large vessels constitutes a well known type of vascular ring

The boy was referred to the department of thoracic surgery Th ductus was ligated and divided in order to divide the vascular ring The boy s condition deteriorated further however and he died 3 days after the operation

At autopsy the vascular and intracardiac anatomy mentioned was found but th- anomalous origin of the left pulmonary artery was also revealed (Fig 3) The tracheal cartilages had a normal horse shoe shape but the tracheal lumen was very narrow The mucous membranes in the trachea and the main bronchi were swollen and covered with fibrinous exudate

DISCUSSION

The diagnosis of vascular rings has been the subject of some recent reviews (2 3) The diagnosis is primarily based on the awareness of these disorders and the findings at roentgen examination of the oesophagus It is manda tory to examine the oesophagus with a small amount of contrast medium because when a large bolus is swallowed the identation in the oesophagus caused by the vessels may be abolished as can also be seen during fluoros copy The examination should also be per formed in a true lateral position when the impression on the oesophagus is to be seen optimally In many cases angiocardiography must be performed to confirm the diagnosis and to evaluate the intracardiac anatomy be fore surgical treatment can be considered

The findings in this patient illustrate that it is possible to have vascular rings at different levels in the same patient One similar case has earlier been described (3) The relative importance of the different vascular rings can however not be settled Maybe the narrow trachea was the most deleterious malforma tion



Fig 2 (*a b*) Angiography with contrast injection into the pulmonary artery (frontal and lateral projection) Extensive atelectasis in the right lung causing dislocation to the right of the heart and mediastinum and distortion of the right intrapulmonary arteries The arrow indicates the proximal part of the left pulmonary artery branching off from the right pulmonary artery

SUMMARY

A fatal case of an infant with a combination of two vascular rings is described One ring consisted of a right sided aortic arch aberrant left subclavian artery and persistent ductus arteriosus the other of an anomalous left pulmonary artery branched off from the right pulmonary artery The importance of a properly performed roentgen examination of the oesophagus is stressed

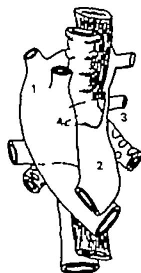


Fig 3 Schematic drawing 1 Ascending aorta 2 Main pulmonary artery 3 Ductus arteriosus 4 Anomalous left pulmonary artery

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CASE REPORT

TRANSVERSE MYELITIS FOLLOWING MUMPS

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Although transverse myelitis is a rare complication of mumps it is relatively well-documented Of 17 examples of myelitis following mumps reviewed by Schead (5) 7 were children under sixteen and in each of these the condition was confined to the transverse form Since then three further cases have been recorded (2 3)

CASE REPORT

A 3 year-old boy one of ten children of an Arab family developed bilateral parotid swelling and fever 7 days before admission On the day of admission his condition suddenly deteriorated with high fever vomiting and retention of urine There was no relevant past or family history

He was apathetic and clearly ill with obvious neck stiffness Tendon reflexes were all diminished the plantar responses being extensor on the right side and equivocal on the left The bladder was distended to the level of the umbilicus Temperature was 37 2 C

The lumbar puncture on admission yielded fluid containing 230 polymorphs/mm The protein level was 48 mg 100 ml glucose 97 mg/100 ml (blood glucose 144 mg/100 ml) Bacterial culture was sterile Blood urea electrolytes Hb and WBC were within normal limits Serum diastase 121 units

Because of the high CSF polymorph count ampicillin was given intravenously for 3 days and continued orally for a further 5 days After several suprapubic aspirations of urine an indwelling urethral catheter was inserted because of persistent retention

A subsequent klebsiella urinary infection was successfully treated with oral nitrofurantoin and neomycin bladder washouts Faecal retention was relieved by enemata

On the fourth day after admission his temperature rose to 39 4 C Neck stiffness persisted Cranial nerve [illegible] normal, but a flaccid paralysis of the legs became manifest accompanied by absence of tendon abdominal cremasteric and plantar reflexes Anaesthesia of the lower half of the body was demarcated by a clear sensory level at the third thoracic segment Lumbar puncture at this time showed 27 polymorphs/mm protein 35 mg/100 ml glucose 56 mg/100 ml In the view of such findings indicative of transverse myelitis prednisolone 10 mg q d s was given for 10 days and tailed off over a further 10 days

On the eighth day no sensory level could be defined and there were weak voluntary movements of the legs All reflexes were still absent Motor improvement continued over the next 2 weeks On the 17th day the knee and ankle jerks were both brisk but abdominal reflexes were still absent On the 22nd day there was clonus at both ankles Urinary retention persisted until the 32nd day when the catheter was removed and urine was passed normally By this time he was able to walk with assistance at which point he was taken home

A verbal report from his parents stated that he was walking normally 3 weeks after leaving hospital and has since remained well

Viral studies No viruses could be cultured from blood stool urine or CSF but mumps complement fixation was positive at 1 256 in the blood and at 1 4 in the CSF on admission

Electroencephalography On the 4th and 20th days was normal on both occasions

Audiometry At 28th day showed no hearing loss

COMMENT

The rarity of transverse myelitis following mumps is in contrast to the high incidence of complicating meningitis and meningoencephalitis Of two recent series of meningoencephalitis complicating mumps totalling in all 256 cases (1 7) only the former paper yielded a

mention of one case of transverse myelitis Schmidt & Hoffman (6) found that the electroencephalogram was abnormal in 49 out of 50 cases of mumps meningoencephalitis in the acute stage The normal EEG in our patient is opposed to an associated basis of encephalitis although the presence of 230 cells/mm³ in the CSF linked to neck stiffness at least suggests meningeal involvement The pyramidal tract signs which appeared in the recovery phase were also found in two out of seven patients discussed by Miller et al (4)

SUMMARY

An example of transverse myelitis complicating serologically proven mumps in a 3 year old boy is described This patient is the youngest as yet reported with the condition

ACKNOWLEDGEMENT

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CASE REPORT

NEONATAL HYPOGLYCAEMIA DUE TO AN ISLET-CELL ADENOMA

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Hypoglycaemia may be idiopathic or induced by leucine The etiology can also be Glycogenosis aglycogenosis congenital deficiency of alpha cells in the islands of Langerhans which probably leads to glucagon shortage (6) galactosaemia fructose intolerance hypoadrenocorticism hypopituitarism hyperinsulinism due to 1) extensive hyperplasia of the islands of Langerhans or 2) a benign or malignant insuloma Congenital islet-cell adenomas seldom cause neonatal hypoglycaemia

CASE HISTORY

A healthy 26 year-old primipara gave birth to the patient a boy The father too was healthy There were no complications during pregnancy On the suspicion that the term was weeks overdue labour was induced medically The child was born in uncomplicated vertex presentation Birth weight was 3 700 g length 52 cm When 19 hours old the patient turned cyanotic there was twitching of the upper extremities opisthotonos and crying Blood glucose at the time was 0.5 mmol/l (9 mg/100 ml) 10% glucose was immediately administered through the umbilical vein, but the blood glucose still remained low 0 8–2 1 mmol l (14–38 mg/100 ml) Hydrocortisone treatment was started on the second day of life 20 mg 6 times a day the dose was then gradually decreased and discontinued entirely after 1 / months On average the blood glucose throughout the treatment was about 2 mmol l (40 mg/100 ml) and the tendency toward convulsions diminished The blood glucose level did not change when the hydrocortisone was discontinued When aged 11 16 days the patient was also given intramuscular injections of glucagon 1 mg twice daily Immediately following the injections the blood glucose rose to maximum 10 mmol l (180 mg/100 ml) but fell again to low values within a few hours Convulsions were checked by intravenous administration of 50% glucose After 9 days of intravenous feeding the patient was given milk mixtures with extra glucose added every 1 /₂–2 hours in an attempt to increase the blood glucose

From the age of 2 /₂–3 / months the patient was fed Alfonim® a substitute for mothers milk which has a relatively low content of leucine since one could not prove that there was no effect of leucine on blood glucose When the child was 2 / months old oral diazoxide treatment was started (7-chloro-3 methyl 1 2 4 benzothiadiazine 1 1-dioxide) 6 25 mg 8 times a day equal to 8 8 mg/kg/24 hours Within 24 hours after beginning the diazoxide treatment the blood glucose rose from approx mmol/l (36 mg/100 ml) to between 3 and 4 mmol/l (54 and 72 mg/100 ml) This level persisted throughout the 7 week long treatment before laparotomy was undertaken on the patient The child had edema one of the side-effects of diazoxide (10) and 25 mg hydrochlorothiazide was therefore administered daily together with the diazoxide with good effect 4 months old the child underwent an explorative laparotomy in order to find the suspected insuloma or glycogenosis Macro- and microscopic examinations of the liver were normal On the anterior surface of the neck of the pancreas a pea sized tumour could be seen and felt it was harder than the rest of the pancreas tissue but was of the same colour It was enucleated The microscopic study showed hyperplasia and hypertrophy of the islets of Langerhans (Fig 1) but the tissue in the sample did not form a coherent adenoma Immediately after removing the tumour the blood glucose was 16 8 mmol/l (307 mg/100 ml) 10 hours later it was 5 9 mmol/l (106 mg/100 ml) (normal) The amount of insulin contained in the excised pancreas tissue was 1 µg/mg tissue which is much higher than one usually finds in pancreas tissue Insulomas generally contain approx 12–60 µg/mg tissue

The postoperative course was uncomplicated The postoperative blood glucose level was the same as during the diazoxide treatment When aged 5 months the child had a single bout of twitching of the extremities and some difficulty in controlling the move

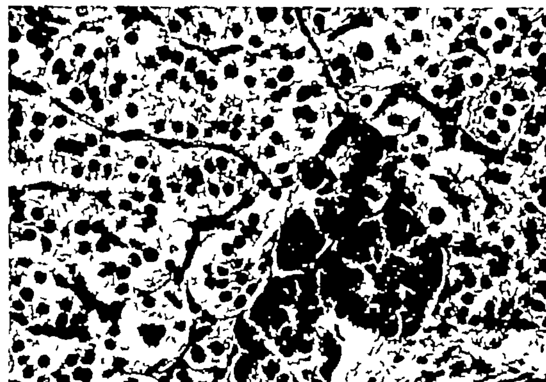


Fig 1 Microscopy of enucleated pancreas tissue (×400) The islands of Langerhans are composed of large pale hypertrophic cells with uniform roundish to oval nuclei The size of the nuclei vary slightly there is no necrosis and no signs of malignancy

ments of his eyes after fasting for 7 hours There has been no recurrence since He is now 1 , years old and normally developed An EEG at 1 year was normal

Special Studies

	I V glucose tolerance test K =	Plasma insulin maximum	Plasma growth hormone maximum
Preoperative	4 5	24 uE/ml	80 ng/ml
Postoperative	3 46	0	10 ng/ml

Preoperative leucine tolerance test Constant low blood glucose rise in serum insulin

Amylo 1 6 glucosidase activity in leukocytes was below the 1% limit for normal values

Amylo 1 6 glucosidase phosphorylase acid maltase (alpha 1 4 glucosidase) glucose 6 phosphate and glycogensyntetase in liver tissue were normal The amount of glycogen in the liver was normal 5 27 %

DISCUSSION

In the present case the patient s hypoglycaemia was discovered during his first day of life Some of the possible causes of hypoglycaemia were gradually eliminated by various tests but by the time the patient was 4 months old glycogenosis or insuloma remained as the possible etiology The reduced amylo 1 6 glucosidase activity in leukocytes pointed toward Cori s type 3 glycogenosis but the patient did not have hepatomegaly

The liver biopsy showed that the etiology was neither glycogenosis nor aglycogenosis The high K value pointed toward hyperinsulinism or idiopathic hypoglycaemia The serum insulin was however never found unusually high and diazoxide treatment was successful In some of previously described patients with congenital islet cell adenoma diazoxide failed to raise the blood glucose (2 11 13) It is characteristic for insulomas that the hypoglycaemia is very resistant to treatment which was also the case with our patient After enucleating the pancreatic adenoma the patient was free of symptoms without any treatment except on a single occasion The growth hormone level in blood fell with a factor 10 Serum insulin was zero a

Table 1 *Previously published cases of islet cell adenoma in newborn infants*

Sex (Ref no) Year of publication	Age at operation	Age at death	Finding at post mortem operation	Type of operation	Postoperative course
F (14) 1947	14 days	6 weeks	Microscopic adenoma	Exploratory	
M (12) 1960		39 hours	Adenoma		
F (1 3) 1961 1962		3 days	Adenoma cerebral edema & haemorrhage		
F (8) 1967	14 months		Adenoma pea sized	75 pancreat ectomy	Severe psychomotor retardation
M (4) 1968	7 / months		Adenoma 8 6 6 mm	Subtotal pan createctomy	Mild psychic retardation
F (11) 1968	7 weeks		Adenoma 4 5 mm	80 % pancreat ectomy	Mild motor retardation
F (5) 1970	14 weeks		Adenoma 5 5 10 mm	Partial pan createctomy	Normal develop-ment
M (7) 1971	4 months		Adenoma 4 mm	75 pancreat ectomy	Facial myoclonia otherwise normal
M (9) 1971	19 days		Adenoma 5 mm	75 pancreat ectomy	Normal develop-ment
M (9) 1971		4 days	Adenoma 10 5 mm		
M (13) 1971	38 days		2 adenomas & adenomatosis	ca 66 pan createctomy	Psychomotor retardation Spasms again 3 years old
M (present patient)	4 months		Adenoma 5 5 10 mm	Enucleation of adenoma	Normal develop ment

fortnight after the operation even during an i v glucose tolerance test This may possibly have been due to the fact that the adenoma s insulin production had impeded the rest of the pancreatic insulin production The pancreas had also been under the influence of diazoxide right until the operation Diazoxide probably also acts by restraining the insulin secretion by the pancreas (10) It is inexplicable why the blood glucose was relatively low at the same time There was probably a good insulin production again when the child was 10 months old The K value was normal for his age at this time

SUMMARY

A full term newborn showed signs of hypoglycaemia which was very resistant to treatment At the age of 4 months the child underwent an explorative laparotomy An adenoma was found in the pancreas and was enucleated The insulin content of the adenoma was extremely high No postoperative complications occurred and the child has since developed quite normally

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CASE REPORT

A GIRL WITH RECURRENT INFECTIONS LOW IgM AND AN ABNORMAL CHROMOSOME NUMBER 1

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Investigations in clinical immunology during the past 20 years had led to the detection of a still increasing number of variants of the classical humoral and cellular immunological diseases This has recently resulted in a new and more modern classification of the immune deficiency states (7)

In the following a girl 2 1/ years of age is described who from the age of 3 months had many recurrent infections caused mainly by microorganisms with a polysaccharide structure In addition to that the patient had slightly fluctuating but constantly low concentrations of IgM in her serum and lacked isoagglutinins On the other hand cellular immunity seemed to function adequately Furthermore we were able to show an abnormal chromosome number 1 in more than 80 of the cells investigated from the peripheral blood

CASE HISTORY

This female patient was born in the 7th of June 1969 after a normal pregnancy and delivery Birth weight was 3 400 g and birthlength was 50 cm Family history revealed several cases of diabetes among her closest relatives The parents were first cousins A 6 year-old sister had bilateral malformations of the urinary tract with recurrent urinary infections

At 3 months of age the patient was admitted to our pediatric department suspected of having meningitis Just before admission she had been treated by her family doctor with penicillin because of a chronic otitis media During most of her hospital stay she had clinical signs of meningitis Her cerebrospinal fluid was examined on several occasions but no microorganisms were cultured The course of the illness was rather protracted and was complicated by 2 attacks of suppurative otitis media

After her initial admission the patient subsequently had a total of 8 admissions to our department She also was admitted to our otologic department on 6 occasions besides being treated by her local otologist several times The overwhelming majority of her infections were caused by *hemophilus influenzae* type b pneumococci and different strains of *Escherichia coli* The various infections are listed in Table 1 together with the microorganisms cultured During her first 2 years of life the patient was anemic and had impaired growth However after having been placed on prophylactic ampicillin at 18 months of age she has shown no signs of infection and is developing normally in every way

IMMUNOLOGICAL INVESTIGATIONS

White blood cell counts As a rule the findings were normal, but during an attack of suppurative otitis media in November 1970 leucopenia and granulocytopenia were noted Lymphopenia was never demonstrated Thrombocyte counts too were normal on several occasions

Serum electrophoresis This was performed on the 19th August 1969 and 21st January 1970 and showed a total gammaglobulin fraction of about 0.5 g/100 ml normal for the patient s age

Quantitative immunochemical determination of immunoglobulins a m Laurell & Weeke (12, 21) The first 2 investigations were performed during a period of severe attacks of suppurative otitis media which were partly of a chronic nature when *hemophilus influenzae* type b was cultured from the middle ear The concentrations of IgG and IgA were then within normal limits for age but IgM was 8 and 7 mg/

Table 1 *List of infections before 1½ years of age*

Type of infection	Number	Culture
Meningitis	1	Negative
Suppurative otitis media	15	Hemophilus influenzae pneumococci streptococci
Laryngitis	5	Hemophilus influenzae
Sinuitis purulenta	4	Hemophilus influenzae pneumococci
Urinary tract infections	2	Escherichia coli
Gastroenteritis	1	Escherichia coli 026

100 ml respectively being approximately 50 below the lower limit for the patient's age A 3rd determination of the patient's immunoglobulins was done 3 weeks after a 4th injection of a standard DPT+polio was given The concentration of IgM was then 23 mg/100 ml while IgG and IgA remained in the normal range When the patient was 2 / years old a 4th determination was done IgM was still low i e 13 mg/100 ml whereas IgG and IgA were 740 and 63 mg/100 ml respectively (all the determinations of the patient's immunoglobulins together with normal values for the age of the patient are listed in Table 2)

Serum isoagglutinins The blood type of the patient was A Rh positive but when she was 16 17 and 19 months old respectively it was not possible to detect anti B isoagglutinins in her serum either by the indirect Coombs technique or by the saline technique at room temperature

Determinations of antibodies

1 Coliantibodies The patient had had a severe attack of gastroenteritis with isolation of *E coli* 026 from the stool She had also had 2 attacks of urinary tract infection with significant growths of *E coli* in the urine Nevertheless she showed no circulating antibodies against a test panel of coli antigens ranging from 01 to 075 including *E coli* 026

2 Hemophilus influenzae antibodies Despite innumerable infections with *hemophilus influenzae* type b the patient had no demonstrable antibodies against this microorganism in her serum Tests for these were carried out by a sensitive hemagglutination technique

3 Polioantibodies 3 weeks after a 4th injection with Salk vaccine composed of polio type 1 2 and 3 serum antibodies were 6 360 and 8 units respectively

4 Diphtheria—and tetanus antibodies 3 weeks after a 4th injection with DPT vaccine diphtheria antibodies were 0 63 units/ml and tetanus antibodies were 0 4[illegible] units/ml serum

5 Typhus antibodies On 2 occasions with an interval of 12 days the patient was given pure typhoid vaccine (0 15 ml) but 16 days after the first injection no antibodies either to H or to O antigens were present in her serum

Stimulation of peripheral lymphocytes with phytohemagglutinin This test was performed when the patient was 2 years old and showed a normal blast transformation of the lymphocytes

1 fluor 2 4-dinitrobenzene (DNFB) stimulation of the skin A sensitizing dose of 10 DNFB solution after 3 weeks followed by a new stimulation with 0 01 molar DNFB solution showed a positive reaction that is to say redness and slight induration of the skin The patient has never received BCG vaccine and did not react to tuberculin

Leucocyte antibodies Neither leucoagglutinins nor leucotoxins could be demonstrated in the patient's serum

Nitroblue tetrazolium test (NBT test) This was performed by a modification of the technique of Park & Good (14) and of Park et al (15) using typhoid vaccine (23) as a stimulator for the hexose monophosphate shunt of the granulocytes and showed a normal occurrence of NBT positive granulocytes

Histological investigations

1 Adenoid tissue This was removed in June 1970 because of the recurrent infections and the presence of lymphocytes and plasma cells was demonstrated the latter however to a lesser extent than normal

2 Bone marrow when the patient was 2 years old showed a normal erythro granulo and lymphopoiesis Some plasma cells were identified

3 Lymph node also removed when the patient was 2 years old showed a considerable number of small lymphocytes in the paracortical thymus dependent areas of the lymph node (16) Plasma cells were also identified

Table 2 *Immunoglobulins and isoagglutinins of the patient*

Date	IgG (normal 400-1 080 mg/100 ml)	IgA (normal 9-115 mg/100 ml)	IgM (normal 13-99 mg/100 ml)	Isoagglutinins
21 10 1970	530	48	8	Absent
4 11 1970	550	49	7	Absent
24 2 1971	430	29	23	Absent
25 12 1971	740	63	13	

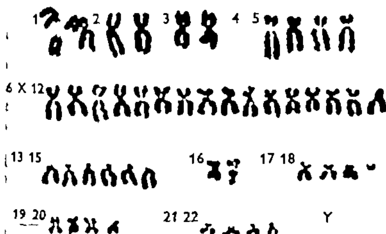


Fig 1 Chromosome picture of the patient showing constriction of chromosome number 1

Family investigations

The father the mother and the 6 year old sister were the only available relatives A quantitative determination of their immunoglobulins was made This showed IgG IgA and IgM levels within normal limits for age although the father s IgM level was relatively low A simultaneous determination of isoagglutinin levels in the same relatives showed normal conditions in the mother and the sister but a low level in the father

Chromosome investigations

The patient s mother and the 6 year-old sister had a normal chromosome analysis The father had a normal male karyotype However one single cell in one of two chromosome studies showed a translocation between 2 chromosomes in the D group

Chromosome analysis in the patient took place on the 3rd of March and on the 8th of June 1971 and revealed a normal female karyotype However a constriction of chromosome number 1 was demonstrated in more than 80% of the investigated cells A reexamination of the patient s chromosomes took place on 9th May 197 this time supplemented with a fluorescein technique The same constriction of chromosome number 1 was found but no further abnormalities of the chromosomes structure could be demonstrated (Fig 1)

DISCUSSION

This girl had recurrent infections mainly caused by polysaccharide microorganism (*hemophilus influenzae* type b pneumococci and *Escherichia coli*) associated with low IgM and no isoagglutinins in serum Furthermore we were able to show a constriction of chromosome number 1 There was a failure to produce antibody to polysaccharide antigens (*Escherichia coli hemophilus influenzae* type b and *Salmonella typhi* O antigen) but a normal antibody response to protein antigens (tetanus diphtheria)

The immunological defect presented in this paper is strongly similar to a group of patients lacking the ability to make antibodies to antigens with a polysaccharide structure This abnormality is mainly described in connection with the Wiskott Aldrich syndrome These boys have varying but constantly low isoagglutinins and IgM in their serum In addition they have chronic dermatitis thrombocytopenia and a progressing reduction of cellular immunity

The probability that the immunological defect in Wiskott Aldrich syndrome is caused by a lack of response to antigens with a polysaccharide structure is supported by the evidence that attempts to immunize the patients with polysaccharide antigens does not (or does only to a slight degree) result in an antibody response (1 4) It therefore seems reasonable to suppose that the low IgM concentration and the lack of isoagglutinins in serum are secondary to a reduced response to poly

saccharide antigens since antibodies to those antigens normally sustain the concentration of IgM in serum The immunological defect is obviously in the afferent limb of immunity i e localisation recognition and processing of antigens, in the proper transfer of the immune message by the effector cells (1 4 5 24) Our patient had a low IgM concentration in serum lacked isoagglutinins, but she did not present skin manifestations thrombocytopenia or reduced cellular immune reactivity

Girls superficially resembling our patient were described by Evans & Holzel (5) and by Blecher et al (2) The patient of Evans & Holzel however had in addition to low IgM and low isoagglutinins impaired cellular immunity and Blecher et al s patient had normal immunoglobulins in serum in spite of a reduced capacity to make antibodies against several antigens Furthermore Kretschmer et al (11) presented a girl with low IgM due to lymphocytotoxic autoantibodies which manifested itself in lymphopenia during acute infections In our patient however neither lymphopenia nor lymphocytotoxic antibodies could be demonstrated

Faulk et al (6) together with Stoelinga et al (20) each described a boy with severe bacterial infections None of these boys had IgM or isoagglutinins in serum—not even after attempts to stimulate them with different antigens These boys seem to belong to a group of patients called dysimmunoglobulinemia type 5 with no IgM formation owing to a lacking production of heavy polypeptide chains connected with IgM Our patient however had constantly low but fluctuating IgM concentrations and resembles in this respect a boy described by Kouvalainen et al (10) who was able to make IgM antibodies The antibody response was short lived however and was as a rule weakly opposed to polysaccharide antigens

The literature concerning connections between immunoglobulin disorders and chromosome abnormalities gives a highly varied picture Hecht (9) and Rudd et al (18) found deletion of the long arm of chromosome number 18 in some patients with low IgM concentration whereas Haddad et al (8) found deletion of the short arm of chromosome number 18 in a patient with the same immune disorder Lisker & Cobo (13) demonstrated chromosome breakage in a patient with ataxia telangiectasia patients who often have low IgA in serum Sellyei et al (19) found chromosome breakage in a mentally retarded patient with no immunoglobulins at all Furthermore Haddad et al (8) found deletion of chromosome number 18 in a patient with low IgM and Christensen et al (3) demonstrated a ring chromosome number 18 in a mentally retarded woman who had no IgM in serum

On the other hand there seems to be a connection between IgM and the X chromosome Thus Rhodes et al (17) were able to show that the IgM concentration in serum was significantly higher in women than in men The authors also found that women with an extra X chromosome had a considerably higher IgM concentration than had normal women Therefore it is very likely that the production of IgM is directed from a locus on the X chromosome and obviously from both X chromosomes which is not in accordance with the Lyon theory that the one X chromosome already in the embryo depresses the genetic expression of the other X chromosome

According to the chromosome abnormality demonstrated in our patient it cannot be entirely excluded that some IgM antibodies for instance antibodies to polysaccharide antigens are directed from chromosome number 1 and eventually modified by the X chromosome This of course can only remain hypothetical and only further investigations especially in Wiskott Aldrich patients can help clarify this condition

SUMMARY

A girl now 3 years of age is described who since the age of 3 months had many often severe infections mainly caused by microorganisms with a polysaccharide structure In

addition she had very low IgM and no isoagglutinins in her serum and furthermore we were able to show an abnormal chromosome number 1 in more than 80 of the cells investigated from peripheral blood The possibility that antibodies to polysaccharide antigens are directed from chromosome number 1 is referred to in this paper

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saccharide antigens since antibodies to those antigens normally sustain the concentration of IgM in serum The immunological defect is obviously in the afferent limb of immunity i e localisation recognition and processing of antigens, in the proper transfer of the immune message by the effector cells (1 4 5 24) Our patient had a low IgM concentration in serum lacked isoagglutinins, but she did not present skin manifestations thrombocytopenia or reduced cellular immune reactivity

Girls superficially resembling our patient were described by Evans & Holzel (5) and by Blecher et al (2) The patient of Evans & Holzel however had in addition to low IgM and low isoagglutinins, impaired cellular immunity and Blecher et al s patient had normal immunoglobulins in serum in spite of a reduced capacity to make antibodies against several antigens Furthermore Kretschmer et al (11) presented a girl with low IgM due to lymphocytotoxic autoantibodies which manifested itself in lymphopenia during acute infections In our patient, however neither lymphopenia nor lymphocytotoxic antibodies could be demonstrated

Faulk et al (6) together with Stoelinga et al (20) each described a boy with severe bacterial infections None of these boys had IgM or isoagglutinins in serum—not even after attempts to stimulate them with different antigens These boys seem to belong to a group of patients called dysimmunoglobulinemia type 5 with no IgM formation owing to a lacking production of heavy polypeptide chains connected with IgM Our patient however had constantly low but fluctuating IgM concentrations and resembles in this respect a boy described by Kouvalainen et al (10) who was able to make IgM antibodies The antibody response was short lived however and was as a rule weakly opposed to polysaccharide antigens

The literature concerning connections between immunoglobulin disorders and chromosome abnormalities gives a highly varied picture Hecht (9) and Rudd et al (18) found deletion of the long arm of chromosome number 18 in some patients with low IgM concentration whereas Haddad et al (8) found deletion of the short arm of chromosome number 18 in a patient with the same immune disorder Lisker & Cobo (13) demonstrated chromosome breakage in a patient with ataxia telangiectasia patients who often have low IgA in serum Sellyei et al (19) found chromosome breakage in a mentally retarded patient with no immunoglobulins at all Furthermore Haddad et al (8) found deletion of chromosome number 18 in a patient with low IgM, and Christensen et al (3) demonstrated a ring chromosome number 18 in a mentally retarded woman who had no IgM in serum

On the other hand there seems to be a connection between IgM and the X chromosome Thus Rhodes et al (17) were able to show that the IgM concentration in serum was significantly higher in women than in men The authors also found that women with an extra X chromosome had a considerably higher IgM concentration than had normal women Therefore it is very likely that the production of IgM is directed from a locus on the X chromosome and obviously from both X chromosomes which is not in accordance with the Lyon theory that the one X chromosome already in the embryo depresses the genetic expression of the other X chromosome

According to the chromosome abnormality demonstrated in our patient it cannot be entirely excluded that some IgM antibodies for instance antibodies to polysaccharide antigens are directed from chromosome number 1 and eventually modified by the X chromosome This of course can only remain hypothetical and only further investigations especially in Wiskott Aldrich patients can help clarify this condition

SUMMARY

A girl now 3 years of age is described who since the age of 3 months had many often severe infections mainly caused by microorganisms with a polysaccharide structure In



Fig 1 The patient at 3 years of age

globin A Both parents were subsequently examined but no haemo lobinopathy was found in either The child has never been anaemic and blood films are normal Subsequently the blood of the child and parents was examined by Miss Patricia Tippett of the M R C Blood Group Unit with the following results

	ABO	MNS	P	Rh	Lu	K	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Do^a
Father	O	MSNs	+	R r	−	−			+	−	+	+	+	+
Mother	O	MSNS	+	$R_1{}^wR_1$		−	+	−	+	−	+	−	+	+
Patient	O	MSNs	+	R_1 r	−	−			+	−	+	−	+	+

At the age of 3 years the child weighs 9 2 kg (Fig 1) She has a small head (maximum circumference 43 cm) with mental retardation and there is marked hypertonicity and adductor spasm causing a poor gait There is epicanthus but the keratitis has cleared away palpebral fissures are level The ears are normal in size and position The base of the nose is flat the nostrils are narrow but there is still posterior choanal stenosis The mandible is small and so is the mouth the palate is high Coarctation of the aorta has not yet been corrected surgically The child vomits frequently There are no hernias The hands and feet are normal Growth is very slow Dermatoglyphic patterns show minor deviation She has had no fits in the past year

Chromosome analysis

This was carried out on three different samples of peripheral blood and on two specimens of skin Chromosome preparations from the first sample were sent to Miss Maureen O Riordan M R C Unit, Western General Hospital, Edinburgh who identified the ring G chromosome

In the blood 114 metaphase plates were counted 17 cells had 46 chromosomes, 80 cells had 45 and 17 cells had 44 Photographic analysis of 75 lymphocyte metaphase plates revealed 7 of 46,XX 12 of 45,XX G and the remaining 6 plates were aneuploid showing random loss of various chromosomes Chromosome banding analysis of 14 of the 45 XX G plates showed the normal chromosome in each to be number 1 (Fig 2) In chromosome 1 the banding affects centromere plus proximal half of the long arms, while the banding in chromosome … is limited to the centromere Our conclusion from these results is that the patient's blood exhibits a mosaic chromosome pattern 46,XX/45,XX 21 in a ratio of 15 46 XX 85 45 XX 21

In the skin preparations 57 metaphase plates were counted 27 cells had 46 chromosomes 22 had 45 and 8 had 44 Photographic analysis of 39 fibroblast metaphase plates revealed 11 of 46 XX Gr and various other karyotypes of aneuploid number showing random loss of chromosomes but generally retaining the Gr fragment This level of random loss has been characteristic of fibroblast cultures in our laboratory We conclude that the skin karyotype is 46,XX Gr

The parents blood showed no chromosome abnormality

DISCUSSION

We have studied published reports of twenty one cases and the present case each of whom had either three autosomes in the "G group or had three "G chromosomes and a "ring chromosome Three of these reports are discarded from further consideration here for the following reasons

Benson et al (2) described a 37 year-old woman in whom only 4 of the leucocyte preparations were 46 XX Gr in type The patient had lymphoedema of the left foot and ankle and no other abnormality Cohen (6) reported a cyclops like monster who died with

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CASE REPORT

A G DELETION SYNDROME ANTI MONGOLISM

H G RICHMOND P MACARTHUR and D HUNTER

From the Departments of Pathology and Paediatrics Raigmore Hospital Inverness Scotland

Partial monosomy of a G chromosome was first reported by Lejeune et al (15) in a child showing an unusual mosaic chromosomal pattern namely 45 XY G /46 XY G minute Since then twenty one examples of partial or complete G group monosomy have been reported

Following the discovery of banding patterns in metaphase chromosomes by the fluorescence technique (4) the two members of the G group were separated by their distinctive fluorescence pattern It has been internationally agreed (12) that chromosome 21 should be identified by its characteristic intense fluorescence banding involving centromere and the proximal half of the long arms as being smaller than number 22 and as the chromosome associated with Down s syndrome

While the particular chromosome associated with mongolism is so defined the chromosome involved in cases of G deletion has not been accurately established It is unlikely that all the reported cases concern the same chromosome and thus the term anti mongolism would be inappropriate in some instances Indeed the essential clinical features associated with partial or complete monosomy of a G chromosome are not yet established and Hall et al (11) discuss the possible genetic variations that may be associated with the clinical syndromes as presently defined

In this communication we describe a child exhibiting partial deletion of G chromosome number 21 The missing chromosome has been identified by its chromatin banding pattern identical to that produced by the fluorescence technique but using Romanowsky dyes The method we have used with minor modifications is that of Seabright (21)

CASE REPORT

L L is the only child of healthy un related parents The child s father aged 31 years has had a chest X ray annually for ten years The mother aged 29 years has had one or two X rays Labour was induced at 41 weeks gestation by the family doctor (Dr R Burnett Thurso) on 2nd November 1968 and there followed a simple vertex delivery of a female child weighing 098 g

During the first 2 weeks the child was difficult to fe d had several cyanotic spells and was referred to the Paediatric Service She had severe posterior choanal stenosis and very narrow nostrils She had also clinical signs of coarctation of the aorta which was subsequently confirmed by cardiac catheterisation (Dr Hamish Watson Dundee) Other abnormal features recognised included micrognathus microcephaly and mental subnormality severe generalised hypertonicity of muscles lightning spasms bilateral keratitis (worse in the right eye) and constantly recurring severe respiratory infections

From birth the child had frequent loose acid stools Appropriate investigations excluded malabsorption syndromes and sweat electrolytes were normal

When the child was nearly 2 years old Professor Lehmann of the M R C Abnormal Haemoglobins Research Unit at Cambridge found that her blood contained 45 Haemoglobin F and a low Haemo



Fig 1 The patient at 3 years of age

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At the age of 3 years the child weighs 9 2 kg (Fig 1) She has a small head (maximum circumference 43 cm) with mental retardation and there is marked hypertonicity and adductor spasm causing scissoring There is epicanthus but the keratitis has cleared away palpebral fissures are level The ears are normal in size and position The base of the nose is flat, the nostrils are narrow but there is still posterior choanal stenosis The mandible is small and so is the mouth the palate is high Coarctation of the aorta has not yet been corrected surgically The child vomits frequently There are no hernias The hands and feet are normal Growth is very slow Dermatoglyphic patterns show minor deviation She has had no fits in the past year

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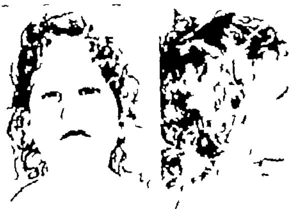


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in hours of birth and in whom leucocyte cul ture showed a mosaic 46 XX/45 XX G Blank & Lorber (3) reported a 6 month old female child who bore a strong clinical resemblance to Down s syndrome but in whom chromo some analysis showed a mosaic of 45 XX G / 46 XX Gr The ring chromosome in this case was very much larger than the other G group chromosomes and the authors are probably correct in suspecting that the ring con tained extra chromatin effectively giving rise to a trisomy of autosome 21

Our review is based upon the remaining nineteen reported cases, including our own pa tient Of these eight cases have been dis covered in the USA (1 9 14 18 19 22 24 25) three in England (5 17 20) two in Japan (7 8) and one each in Finland (10) France (15) Sweden (11) Jamaica (23) South Africa (13) and Scotland (present case) Eleven were female and eight were male Nearly all were mature at birth and born at or about term to parents in the 20 to 30 years age group

Cytogenetic analysis showed 10 patients with a 46 Gr karyotype 4 patients with a 45 G / 46 Gr karyotype 4 patients with a 45 G karyotype and 1 patient with a 46 XY Gr B inv (p−q+) karyotype All patients old enough to test have been mentally retarded and in thirteen the head size was small In four patients the head was of normal size Somatic growth has been slow in twelve pa tients and normal in four Minor skeletal ab normalities have been present in seven chil dren

The ears are large and/or low set in four teen cases and are normal in three cases in cluding our own patient The eyes are often abnormal The palpebral fissure slopes down wards and outwards in nine children epi canthus is present in four cataract in three keratitis in three blepharochalasia and eccen tric pupils in one and in three the eyes are normal The nose has been prominent in five and broad and flat in ten The mandible is small in ten patients Our patient alone has choanal stenosis The mouth is small or nor

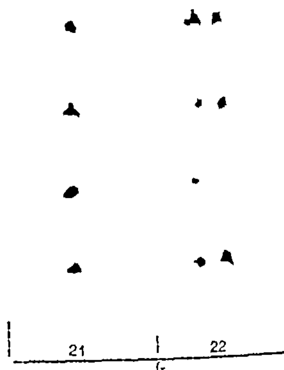


Fig 2 The G group chromosomes stained by the chromatin binding technique identifies the missing chromosome as number 21

mal A heart murmur was present in seven with one child having aortic stenosis two with coarctation of the aorta and other anomalies and one with ventriculoseptal defect and pul monary stenosis Nine children had a normal heart Persistent vomiting was troublesome in nine children of whom three had pyloric stenosis Five children have suffered convul sions

Four of the nine male children had hypo spadias and two had renal agenesis One child had a cleft palate and hare lip and eight had a highly arched palate Four children had thrombocytopenia and one other had a bleed ing tendency with normal platelets Our pa tient had 45% foetal haemoglobin in her circulating red cells

Blood groups of patients and their parents were studied (1 8 10 14 20 24 25) but no genetic markers on the G group chromo some were discovered The results of our blood group studies in our case are also nega tive

Dermatoglyphic patterns show minor deviations only in all reported cases to date

Hoefnagel and his colleagues (14) suggested that there were two clinical types of response in the G deletion syndrome and this has been considered by other authors (20 25) and has been further developed by Warren & Rimoin (24) who claim to be able to separate reported cases into one or other of the two types they recognize G deletion syndrome type I (antimongolism) and G deletion syndrome type II The principal difference between these syndromes is that in the first the child is hypertonic while in the second he is hypotonic It has been suggested that type I is due to deletion of a 21 chromosome while type II is due to deficiency of chromosome 22 but this is not yet proved Attempts have been made in the past to differentiate autosomes 21 and 22 by culture of cells using tritiated thymidine and autoradiographic studies Some authors doubt if this technique is useful (25) while others claim that it showed the deletion in their case to be a 21 chromosome (8 10 22) In our case a child showing marked hypertonicity we have identified the missing or partially deleted G chromosome as number 21 using the chromatin banding technique It is important that cases should be analysed with this technique so that the question of two types of "G deletion syndrome can be placed on a scientific basis

It is worth noting that on the evidence of other workers findings in plant and animal tissues Mittwoch (16) has suggested that the delayed growth in trisomic conditions is due to the increased DNA content of the nuclei which leads to retardation of the mitotic cycle In our case which is established as a partially deleted "G the retardation of growth is comparable to the retardation found in trisomy G which lends no support to her hypothesis

SUMMARY

The clinical features and laboratory findings in patients with deletion of a chromosome from the G group are described with details of a new case which reveals hitherto unrecognized features Special studies using the chromatin banding technique have shown that the patient's tissues have partial or complete deletion of a chromosome 21

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PERINATAL MORTALITY

FOLKE PETTERSSON, STIG MELANDER and DAGMAR LAGERBERG

From the Department of Social Medicine (Head: Ragnar Berfenstam) and the Department of Gynaecology and Obstetrics (Head: Carl Gemzell), University Hospital, Uppsala, Sweden

The Swedish figures on perinatal mortality show a significant decrease from 1910 to 1970. The decline during this period in the overall perinatal mortality rate is generally accepted as reflecting the combined effect of comprehensive factors such as advances in medicine, improved maternal and child care and better medical facilities as well as more favourable economic and environmental conditions. In addition, the change in family size during the last 50 years towards one- or two-child families has certainly to some degree contributed to the low mortality figures. This signifies that high risk multiparous elderly mothers nowadays constitute a smaller proportion than before of the group of pregnant women. Certain factors, however, may be supposed to work in the opposite direction, e.g. the increasing number of diabetic pregnant women and the mounting number of pregnancies occurring after hormonal treatment in previously infertile women. The higher hospitalization rate in connection with deliveries may also lead to an improvement in reporting live births and thence to a relatively more complete registration of deaths in early infancy. Thus the mortality rate may increase artificially, and this phenomenon is probably what can be seen in Fig. 1, relating to the years 1920–1940, during which period the hospitalization rate for deliveries increased from about 20% to nearly 100%.

A question still requiring an answer is whether or not and to what extent the reduction of high risk pregnancies by the increased number of legal abortions may have contributed to the favourable figures on perinatal mortality. In any case, it seems fair to state that many high risk pregnancies occur among aborting women. This is due to factors such as debilitating diseases, exposition to rubella or radiation during pregnancy, or to the fact that the woman has had a malformed child previously, is a multipara, or alternatively is very young.

Even though recent decades have shown a decline in perinatal mortality, still nearly 20 out of 1 000 children die during the perinatal period. Therefore an investigation of what factors lie behind these deaths is still justified, especially in cases where the general factors mentioned above do not seem to have been predisposing. These remaining causes of perinatal death, which thus cannot be associated with general factors, are often related to factors affecting the development of the child in utero and to conditions affecting the delivery. Although these factors and conditions are not easily identified, a great number of observations have been published showing a relationship between conditions associated with pregnancy and delivery and characteristics of the pregnant women on the one side and perinatal

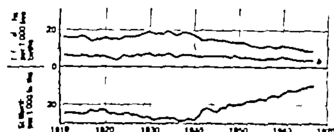


Fig 1 Stillbirths and early infant deaths during the first week of life Sweden 1911-1967 (a) stillbirths (b) deaths during the first 24 hours (c) deaths during the first week of life

mortality on the other Thus the following factors, among others have been found to correlate with a higher than average perinatal mortality viz. male sex (3 15 28 32) low birth weight (2, 10 28 32 33) young and old mothers (3 26 28 30, 32) primiparous and multiparous state (3 25 26 30 32) previous reproductive history of foetal wastage (3, 26 28 30) toxaemia (3 15, 25 28) antepartum bleeding (3 15 28 30) maternal diabetes (21 28) maternal infection (6 13 14 25) maternal anaemia (30) Conflicting opinions exist about the influence of smoking on perinatal mortality (4 5 8 12 18 27)

Other factors known to be associated with a higher than average perinatal mortality are prolonged pregnancy especially among primigravidae (3 16 17 33) blood incompatibility (4 30) uterine inertia, dysfunctional labour prolonged labour (3 28 30) premature delivery (3 33) breech presentation (3 15 30) cord complications (28 32) foetal asphyxia (4 32) caesarean section (3 28) A major cause are congenital malformations (4 25 32)

In addition some socio economic and geographic factors have been shown to influence perinatal mortality Thus a higher perinatal mortality has been found in lower socio economic groups (3 26 30 32) Illegitimacy has also been interpreted as connected with a high perinatal mortality (15 20 30 32) but it should be noted that a considerable part of the registered higher risk of perinatal death in children of unmarried mothers has been proved to be due to false conclusions deriving from methodological errors (23) Ultimately it may be mentioned that deficient antenatal care is often connected with higher rates of perinatal mortality (3 30 32)

In this connection it ought to be emphasized that it may sometimes be difficult to isolate the exact influence of one single variable on perinatal mortality although this factor may be known to be correlated with a higher than average risk of perinatal death Often there will be a covariation between different factors such as for instance age and pregnancy order age and marital status age socio-economic group and pregnancy order etc Difficulties will then arise in the interpretation of simple correlations Furthermore, several factors will cooperate in bringing about the end result—perinatal death Some trials have been made to solve these difficulties by means of multivariate analyses (e g 8) There is of course also the possibility of solving these problems by a far reaching division of the material into subgroups A new problem will then arise, as the subgroups will tend to become too small to permit reliable statistical analysis There exist also other methods of solving the problems connected with multifactorial analyses, e g various standardizing procedures These methods however will also prove impractical when many factors are involved

PRESENT STUDY

Definition of Perinatal Mortality

Perinatal mortality is defined as th number of still born children (29 weeks of gestation or more)+live born children dying within 7 days of delivery related to the total number of children born during the period under study in the same population Statistical aberrations may arise from the use of the arbitrary cut off point of 29 weeks of gestation as the dividing line between foetal deaths and stillbirths but even at this moment it is still the accepted method of calculating perinatal mortality Moreover complete data are not always available on early intra uterine foetal deaths occurring for instance between the 20th and 28th week of gestation

Material and Methods

The material of the present study consists of a consecutive series of deliveries from November 1967

Table 1 *Set of variables used in the multiple regression analysis*

1	Age –
2	(Age –)
3	Pregnancy order 1 = 1 others = 0
4	Breech delivery primipara
5	History of previous abortion = 1 others = 0
6	Blood group incompatibility = 1 others = 0
7	Non-smokers = 1 others = 0
8	Breech delivery = 1 others = 0
9	Delivery > 14 days prior to expected date = 1 others = 0
10	Boy = 1 girl 0
11	Placenta previa or abruptio placentae prematura = 1 others = 0
12	Eclampsia praeclampsia or toxaemia = 1 others = 0
13	Birth weight < 1 000 g = 1 others = 0
14	Birth weight < 2 500 g 1 others = 0
15	Congenital malformation = 1 others = 0

to the end of December 1969 from the University Hospital of Uppsala. Also either 7 190 single births have been examined (for technical reasons twin births were excluded) About 100 variables were coded and transferred from the ordinary delivery records to an optically readable record which was thereafter further analysed by computational methods

The series was analysed by means of a multiple regression analysis using the equation Y = a + b x + bx + bx + As the dependent variable (Y) was used the alternative perinatal death = 1 living child = 0 As independent variables different sets of variables were used including age of mother pregnancy order previous history of abortion Rh immunization infection during pregnancy smoking habits toxaemia of pregnancy breech delivery gestational age sex of child congenital malformations birth weight immigrant mother lack of supervision during pregnancy use of certain drugs in connection with delivery (e g Mependine® and Librium®) caesarean section and also different combinations of variables for instance breech delivery in primipara

The multiple regression analysis was performed by the standard computer program BMD03R Out of the above mentioned independent variables the age and the pregnancy order variables were taken in square The pregnancy order variable was also divided into pregnancy order 1 against all other cases After testing different sets of variables and rejecting those without explanatory value the set of variables given in Table 1 was definitely adopted

In a second approach a computer program for selecting optimal combinations of explanatory variables (Automatic Interaction Detector—AID) was used (1 79)

Traditional multiple regression analysis constitutes a powerful tool for analysing social survey data but may be difficult to use because of problems in constructing an appropriate regression model The Automatic Interaction Detector (AID) computer program was developed as a response to this need The underlying question the program seeks to answer is Which explanatory variables and which combinations of these variables are important for reducing the variance in the dependent variables?

In order to fit the material into the scope of the AID program it was primarily split into three groups primiparous women secundiparous women and women with parity three or more Within each of these groups an analysis was made using perinatal death as the dependent variable Independent variables or predictors were the following age group blood group incompatibility smoking urinary tract infection caesarean section breech delivery delivery later than 14 days after expected date delivery earlier than 14 days prior to expected date toxaemia, weight groups (< 1 000 g < 1 500 g < 2 500 g) and congenitally malformed child

RESULTS

Multiple regression analysis

The overall perinatal mortality is 1 52 The analysis shows a coefficient of determination amounting to 0 2245 The regression coefficients and computed *t* values are given in Table 2

As can be seen from Table 2 the perinatal mortality rate is higher for the children of the youngest and the oldest mothers Immunization congenital malformation breech delivery and toxaemia also give a higher risk of perinatal death Low weight babies and those with a low gestational age too run a higher risk of dying during the perinatal period

Table 2 *Results of multiple regression analysis*

Variable no	Regression coefficient	Computed *t* value
1	−0 0001	−0 1
2	0 0001	3 0
3	0 0008	0 2
4	−0 0609	−3 3
5	0 0002	0 1
6	0 0268	3 8
7	0 0059	2 2
8	0 0696	5 1
9	0 0370	5 3
10	0 0027	1 0
11	0 0156	0 8
12	[illegible]	3 6
13	0 6608	26 7
14	0 1298	16 4
15	0 0358	8 9

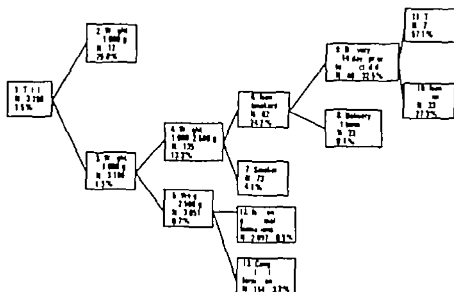


Fig 2 Predictor tree for perinatal deaths Primiparae The percentage figures denote perinatal deaths per 100 live births

Selection of optimal combinations of explanatory variables

The predictor trees for perinatal deaths are shown in Figs 2, 3 and 4 It can be seen from Fig 2 that 12 out of a total of 3 198 primiparous women had babies weighing less than 1 000 g whereas consequently 3 186 primiparous women had babies weighing more than or equal to 1 000 g 75 • of the 12 low weight babies died perinatally This was the case for only 1 3 of the babies weighing at least 1 000 g As an example of the interpretation of a predictor tree it can also be inferred from Fig 2 that an especially high risk of perinatal death is inherent in the situation of a primiparous, non smoking mother with toxaemia delivered earlier than 14 days prior to the date expected and having a child with a birth weight of 1 000–2 000 g This may be contrasted to a primiparous mother with an infant showing the combined factors of a birth weight exceeding 2 500 g and absence of congenital malformations (ultimate mortality rates 57 1 and 0 5 respectively)

DISCUSSION

The overall perinatal mortality rate found in this series 1 52 is rather low compared with figures from several other countries (25) In comparison with the figures on perinatal mortality from other countries, however it must be added that the proportion of low weight babies (prematures) is only 4 4 in the present series, whereas series from many other countries give figures on prematures amounting to about 10 This difference must be kept in mind in discussions concerning our low figures on perinatal mortality

It is not surprising to find that the same high risk groups were detected in the present series as have been pointed out by other authors As could be expected breech delivery Rh incompatibility congenitally malformed infant low weight baby etc proved to be factors associated with higher risks of perinatal death The somewhat unexpected finding that breech deliveries among primiparous women give a lower risk of perinatal death than other deliveries can probably be explained by the eager interest and considerable medical efforts devoted to this group of mothers usually regarded as a high risk group The contradictory finding that smoking women s babies suffer fewer perinatal deaths than those of non smoking women can probably be explained by the fact that the crude dividing of our series in gestational age for each group of comparison will favour the babies of smoking mothers These babies are more mature with the same birth weight as those of the non smo women It is a well known fact that ci

ble 3 *Analysis of perinatal deaths in the "low risk group" among primiparous women according to AID analysis*

e	Age of mother	Maternal health	Pregnancy	Delivery	Infant
	38		Prolonged	Induced with oxytocin. Vacuum extraction	Intrapartum anoxia
	23			Prolonged labour (>48 hours)	Intrapartum asphyxia due to aspiration
	19			Precipitate labour	Intrapartum asphyxia
	0			Premature separation of placenta	
	31				Weight 2 900 g. Death 2 days prior to delivery. Cause unknown. Autopsy: signs of asphyxia
	33	Previous abortion			Weight 2 500 g. Strangulation by the umbilicus
	18	Uterus bicornis	Prolonged	Repeated attempts to induction with oxytocin	Death just prior to Caesarean section
	27	Hereditary haemorrhagic diathesis			*Massive pulmonary* haemorrhage probably due to hereditary disease
	21			Precipitate labour	Asphyxia post partum
	18			Precipitate labour	Asphyxia intra et post partum
	35	14 days prior to delivery treated with penicillin for upper respiratory tract infection			Bacteria cultivated from various foetal organs
	25			Breech presentation. Cervical spasm	
	20				Weight 2 625 g. Death 4 days prior to delivery. Cause unknown. Bacteria cultivated from various foetal organs
	4				Weight 2 880 g. Death some day prior to delivery. Petechial bleedings. No bacteria cultivated
	7		Toxaemia of pregnancy. Hypertonic uterus	Caesarean section	Subarachnoidal bleeding

Table 4 *Analysis of perinatal deaths in the low risk group among secundiparous women according to the AID analysis*

Case no	Age of mother	Maternal health	Pregnancy	Delivery	Infant
1	41	Second late primipara	Toxaemia		Death 2 days prior to delivery Cause unknown
2	29	Previous pregnancy wastage due to immunization	Rh incompatibility with immunization		
3	19				Foetal pneumonia
4	27			Difficult breech delivery	Foetal intraventricular haemorrhage
5	30	Previous jaundice of pregnancy	Jaundice of pregnancy Oedema of pregnancy		Death some day prior to delivery
6	24		Acute poly hydramnios		Death prior to delivery
7	29			Induced with oxytocin Hyper tonic state of uterus during delivery	Massive pulmonary haemorrhage

smoking during pregnancy reduces the birth weight without shortening the gestational length (22)

The same circumstances which are re sponsible for perinatal death may also cause damage to surviving children It has been stated that when infant mortality is high the number of infants who are on the verge of dying is even higher These infants are the ones who are subjected to adverse factors re lated to the process of brain growth and pre sumed educability and their mothers are sup posed to have the same characteristics as those whose children suffer perinatal death It is

Table 5 *Analysis of perinatal deaths in the low risk group among pluriparous women according to the AID analysis*

Case no	Age of mother	Maternal health	Pregnancy	Delivery	Infant
1	25				Death 5 days prior to delivery
2	32	Viral pneumonia (maternal death 14 hours after delivery)			Intrapartum asphyxia
3	33		Toxaemia of pregnancy		Death 48 hours prior to delivery
4	39	Upper respiratory tract infection treated with penicillin	Generalized pruritus		Petechial bleedings No bacteria cultivated from foetal organs
5	41	Symptoms of upper respiratory tract infection not treated			Bacteria cultivated from various foetal organs

therefore of great importance to try to find the relevant causes of perinatal death in order to eliminate these risk factors if possible The high risk groups are found in this study to be the expected conventional ones and a number of preventive measures are already in work against risk factors known to characterize these groups As an instance of this may be mentioned the prevention of Rh isoimmunization by administration of immunoglobulin anti D *Measures* are also attempted to prevent or postpone a threatening premature delivery by pharmacological arrest of premature labour by e g Isoxsuprine Ethanol or Orciprenaline (9 11 19) On the other hand one of the major causes of perinatal death—congenital malformations—is at present certainly unpreventable in most cases Probably a better knowledge and understanding of teratogenic mechanisms will make it possible to avoid the birth of a number of malformed infants Early antenatal intra uterine diagnosis and a subsequent interruption of pregnancy in selected cases of grave malformations will also play some role

Since the coefficient of determination in the multiple regression analysis amounts to 0 2245 only it follows that a large part of the variation cannot be explained by the set of variables chosen In other words even if factors such as breech delivery Rh incompatibility congenital malformation low birth weight etc are really associated with a high risk of perinatal death they are not capable of contributing much to the explanation of the overall perinatal mortality This is in fact what can be expected since many circumstances responsible for deaths in the perinatal period arise from conditions which are not easily caught in simple variables From a purely obstetrical point of view some approach other than division of the material into high vs low risk groups may therefore prove more fruitful in the analysis of perinatal mortality A study in detail of individual cases of foetal deaths in low risk groups may be more profitable Such a situation may arise for instance in a primiparous woman with a baby weighing more than 2 500 g and displaying no congenital malformations We may put the question in this way Why did the baby die under these circumstances which seem to have been favourable to its survival?

In the AID analysis of the children of primiparous women the combination of birth weight over 2 500 g and absence of congenital malformations gave the minimum risk of 0 5% children perinatally dead (Fig 2) This group consists of 2 897 babies The perinatal deaths occurring among these low risk deliveries are characterized individually in Table 3 It can be seen that the majority of babies died in a picture of asphyxia or anoxia In two cases there were prolonged pregnancies with induction of labour by oxytocin and ultimately complicated deliveries In three cases precipitate labour occurred whereas in one case the mother had been treated for an upper respiratory tract infection and bacteria could be cultivated from various foetal organs In other cases foetal death was associated with expected risk factors such as premature separation of the placenta breech delivery toxaemia with hypertonic uterus factors which ought to be found also in low risk groups Some of the individual deaths in low risk groups must of course be associated with known factors of importance to foetal death Similarly Tables 4 and 5 give support to the standpoint that induction of labour by oxytocin and maternal respiratory tract infection may be adverse to foetal welfare

From Tables 3 4 and 5 it can be inferred that repeated attempts towards induction of labour by means of oxytocin should be avoided Caesarean section may be a safer method of delivery to the baby if a first attempt at induction has failed This holds true especially for primigravidae and grand multigravidae It has been emphasized (24, 31) that primigravidae and grand multigravidae are more likely to develop abnormal uterine action with concomitant foetal distress following induction than secundigravidae and tertigravidae

Table 4 *Analysis of perinatal deaths in the "low risk group" among secundiparous women according to the AID analysis*

Case no	Age of mother	Maternal health	Pregnancy	Delivery	Infant
1	41	Second late primipara	Toxaemia		Death 2 days prior to delivery Cause unknown
2	29	Previous pregnancy wastage due to immunization	Rh incompatibility with immunization		
3	19				Foetal pneumonia
4	27			Difficult breech delivery	Foetal intraventricular haemorrhage
5	30	Previous jaundice of pregnancy	Jaundice of pregnancy Oedema of pregnancy		Death some day prior to delivery
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5	41	Symptoms of upper respiratory tract infection not treated			Bacteria cultivated from various foetal organs

than children of corresponding contrasted mothers a finding which may at first seem surprising but which can probably be given a natural explanation

The selection of optimal combinations of explanatory variables gave predictor trees which can be utilized for determination of favourable and unfavourable combinations of factors associated with perinatal death Some individual cases of foetal deaths were analysed in detail The study ends in a discussion of possible preventive measures to be taken in order to prevent perinatal deaths in the future

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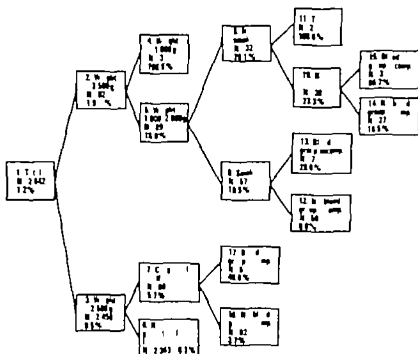


Fig 3 Predictor tree for perinatal deaths Secundiparae The percentage figures denote perinatal deaths per 100 live births

A second conclusion is that precipitate labour should be arrested and treated, if possible by for instance pharmacological relaxation of uterus by Orciprenaline (19), or by caesarean section Respiratory tract infections cannot be treated other than symptomatically in many cases but a pregnant woman should at least try to avoid contact with infected persons

Finally it should be pointed out as a remarkable finding that although the series analysed in this study included 25 cases of diabetic mothers non of their offspring was shown to have suffered perinatal death

SUMMARY

From November, 1967 to the end of December 1969 perinatal deaths among 7 190 consecutive single births in the University Hospital of Uppsala Sweden were analysed in a study using multiple regression analysis and a program for selecting optimal combinations of explanatory variables

The overall perinatal mortality rate, expressed as stillbirths and early infant deaths per 1 000 births was found to be 15 2 the coefficient of determination was 0 2245 A significantly higher perinatal mortality rate was found for the children of the youngest and the oldest mothers of mothers with immunizations, breech deliveries toxaemias short gestational periods, and for low weight and congenitally malformed children Babies of primiparae with breech deliveries and of smoking mothers showed lower perinatal mortality rates

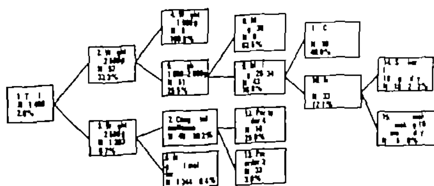


Fig 4 Predictor tree for perinatal deaths Tertiparae or more The percentage figures denote perinatal deaths per 100 live births

Acta Pædiat Scand 62 231–240 1973

STUDIES ON GROWTH HORMONE SECRETION IN A PATIENT WITH THE DIENCEPHALIC SYNDROME OF EMACIATION

A HÄGER and J I THORELL

From the Department of Paediatrics University Hospital Linköping and the Isotope Laboratory General Hospital Malmö Sweden

The diencephalic syndrome of emaciation was first described by Russell in 1951 (30) since when several cases of the syndrome have been reported (1 2 8 11 15 16 38) The cardinal features presenting in infancy or early childhood are extreme emaciation despite an adequate food intake euphoria and motor hyperactivity large hands and feet pallor without anemia and often nystagmus This syndrome is associated with a tumour usually situated in the anterior hypothalamus and the floor of the third ventricle In some of the cases on record (3 9 12 31 34) the level of the plasma growth hormone has been found to be raised This paper describes a typical case of the diencephalic syndrome of emaciation followed for 2 years and in which the growth hormone studies revealed a new type of growth retardation with high levels of plasma growth hormone

CASE REPORT

A. S female born April 4th 1969 was admitted to the Paediatric Department at the age of 6 months because of lack of increase in weight She was the second child of healthy non-consanguineous parents Delivery had been normal after an uncomplicated gestation Birthweight 4 110 g, crown-heel length 52 cm Development was normal during the first 5 months, at the end of which the patient weighed 6 750 g Unfortunately height had not been recorded at the routine examinations at the Child Welfare Centre At the age of 5 months she began to vomit occasionally and to lose weight The parents also noticed abnormal eye movements On admission the patient was strikingly emaciated Bodyweight 6 030 g height 72 cm circumference of the head 43 cm She was pale but otherwise appeared happy alert almost euphoric and was permanently hyperactive except during sleep Examination of the eyes revealed rapid oscillating nystagmoid movements in the horizontal plane The eye grounds were normal Otherwise the neurological examination revealed nothing remarkable Her hands and feet were unusually large Routine analyses of blood and urine gave normal values Neither did serum protein electrophoresis EEG X rays of the upper gastrointestinal tract and the skull d-xylose absorption test and determination of the chymotrypsin content in faeces reveal anything abnormal Analyses of the cerebrospinal fluid on two occasions gave high values 109 and 230 mg/100 ml Because of nystagmus and her large hands and feet the possibility of a diencephalic tumour was considered Pneumoencephalography at the age of 7 months demonstrated a large mass extending into the anterior hypothalamus and the posterior portion of the third ventricle (Fig 1) Craniotomy revealed a large tumour involving both optic nerves and the chiasma Microscopy showed the tumour to be an optic glioma The tumour was inoperable

In spite of an almost normal appetite the child lost weight to 5 200 g during the following months and her height remained unchanged (72 cm) Yet psychomotor development was normal Judging from her behaviour vision appeared unimpaired without further deterioration At 10 months of age she was extremely emaciated Soft tissue radiograms of the legs showed complete absence of subcutaneous fat (Fig 2) The further course was rather stationary apart from temporary deteriorations of her general condition usually in connection with infections of the respiratory or the gastrointestinal tract X ray examination of the skull at 2 years of age revealed expansion of the tumour with signs of increased intracranial pressure Neurological examination at 2 ½ years of age nevertheless still showed nothing re

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In spite of an almost normal appetite the child lost weight to 5 200 g during the following months and her height remained unchanged (72 cm) Yet psychomotor development was normal Judging from her behaviour vision appeared unimpaired without further deterioration At 10 months of age she was extremely emaciated Soft tissue radiograms of the legs showed complete absence of subcutaneous fat (Fig 2) The further course was rather stationary apart from temporary deteriorations of her general condition usually in connection with infections of the respiratory or the gastrointestinal tract. X ray examination of the skull at 2 years of age revealed expansion of the tumour with signs of increased intracranial pressure Neurological examination at 2 / years of age nevertheless still showed nothing re



Fig 1 Pneumoencephalogram (lateral view) at 7 months of age The tumor is indicated by arrows

markable apart from the impaired vision and her clinical condition was essentially unchanged She could walk by herself and talk normally for her age

Trials of treatment (Fig 3)

Dexamethasone was given at the age of 1 year in a dose of 1 mg a day for 3 weeks and _ mg a day for another 3 weeks A temporary improvement of appetite was noticed but height weight and general condition remained unchanged

Radiotherapy with 3 950 rad from a 6 MV linear accelerator was given for 43 days at the age of 15 months This was followed by a slight improvement in the general condition and bodyweight increased by 500 g

Human growth hormone (HGH) (Crescormone® Recip Stockholm Sweden) 2 mg was given intramuscularly twice a week for 10 weeks No change occurred in height weight or clinical status

Insulin Lente (Novo A/S Copenhagen) 6–10 IU was given subcutaneously once a day for 7 weeks The therapy was withdrawn because of a tendency to develop hypoglycaemia During this period the child increased in height and gained from 6 200 to 7 200 g

METHODS

Clinical studies

Unless otherwise stated the investigations were performed when the child was 8 to 22 months old during which time her condition height and weight remained stationary (Fig 3) Height was always measured with the patient in recumbent position All tests were performed after an overnight fast An indwelling catheter was inserted into one antecubital vein Because of the restricted availability of veins for puncture both the injection of test substances and the collection of blood were made through the same catheter Care was taken to avoid contamination and the first portion of collected blood was discarded

Oral glucose tolerance test was carried out with 100 ml of 10 glucose administered by means of a gastric catheter

Intravenous glucose tolerance test was done with 0 5 g glucose kg bodyweight In the *insulin tolerance test* 0 1 IU kg bodyweight was given i v

Nitrogen retention test A milk formula gruel (Barnvalling Semper 800 ml a day) was given for ? weeks The child tolerated this diet well On days 8–13 of this period 1 mg HGH was given i m once a day Urine was collected on days 5–7 and on day 14 For technical reasons it was not possible to collect urine every day

Reserpine was administered i m in a dose of 2 5 mg Blood was collected before and _ and 5 hours after the injection

Chlorpromazine was given i m and orally in a total dose of 15 mg in the course of one day It induced somnolence for 12 hours Blood was collected on the day before and on the day of administration A third blood sample was obtained during the following night when the child was abnormally somnolent



Fig 2 The patient (left) at 10 months of age together with a normal child of the same age

Induction of sulfation factor was performed with 2 mg HGH im on each of 2 consecutive days Blood was collected the day before and the day after the injection

Control subjects

Plasma samples were obtained from 7 age matched healthy children after an overnight fast

Analytical methods

Growth hormone was determined by radioimmunoassay on unextracted plasma with either buffer flow chromatography (37) or double antibody precipitation (36) Both methods gave fully comparable results The concentration of plasma growth hormone is expressed in terms of a highly purified preparation, prepared by AB Kabi Stockholm according to Roos et al (29) 1 mg corresponds to 2 U of the WHO 1st Int. Reference Preparation 66 217 The plasma was tested for growth hormone antibodies by performing the buffer flow assay without addition of growth hormone antiserum to the tubes Insulin was determined by radioimmuno assay with ethanol precipitation of antibodies (14)

TSH FSH and LH were determined by radioimmuno assays (6 25 7) Sulfation factor (Somatomedin) was determined by a method described by Hall (7 13)

Blood glucose was determined with a glucose oxidase method (39) and urea in the urine as modified from Skeggs (33) Free fatty acids were measured colorimetrically (71) and glycerol enzymatically (70)

Plasma amino acids were analysed with a Jeol 5 AH program modified from Benson et al (5)

RESULTS

Clinical data pertinent to the metabolic and endocrine status are given in Tables 1 and 2 and Fig 3 Skeletal and dental age assessed on various occasions were always normal

The basal concentration of growth hormone in plasma was constantly increased although the height of the patient did not increase by more than 3 cm within 16 months The mean value in fasting samples collected on 9 different occasions when the patient was not receiving any treatment was 35 ng/ml (range 13–50 ng/ml) In the control group the mean fasting concentration was 1 5 ng/ml (range 0 5–3 5 ng/ml) After 2 years of age the fasting level tended to decline with a mean basal level at 2 1/ years of age of 9 ng/ml As shown in Table 2 the various tests used to modify the high levels of the growth hormone including oral and intravenous glucose tolerance tests did not reduce plasma growth hormone to normal levels though a moderate decrease was noted Insulin induced hypoglycaemia did not raise the level No effect was recorded by treatment with reserpine dexamethasone and chlorpromazine Following radiotherapy the concentration of growth hormone tended to fall but 1 month later it recovered pre treatment level

Sulfation factor activity was rather low The baseline value of 0 70 was higher than the highest value given by Hall for hypopituitary children (13) The value (0 42) found after injection of HGH was in the hypopituitary range Thus HGH of exogenous origin could not induce sulfation factor activity

Administration of HGH had no effect on nitrogen excretion measured as urea in the rather stationary the patient s condition re



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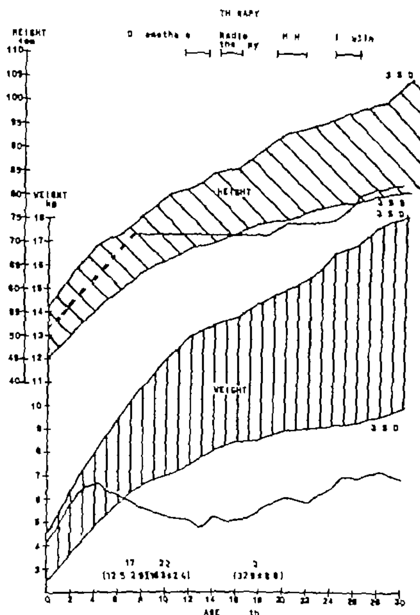


Fig 3 Height and weight development of the patient in relation to normal children Trials of treatment indicated on top Number of ossification centers in the right half of the body is given at the bottom (figures within brackets denote mean ±S D for normal girls)

produce any demonstrable relief The short term tests with reserpine and chlorpromazine urine Long term treatment with HGH did not induce any increase in rate of growth

As shown in Table 3 the plasma concentrations of individual amino acids were essentially normal, with no signs of protein deficiency

DISCUSSION

The case reported in this paper presented the typical features of the diencephalic syndrome of emaciation The histological diagnosis of an optic glioma *confirmed* the clinical diagnosis The course of the disease resembled that in many of other cases on record (2 11) i e did not lower the plasma growth hormone levels and were therefore not tried for long maining unchanged from 6 months to 2¹/ years of age

It is doubtful whether the course was affected by the treatments given Several types of therapy were tried (Fig 3) essentially in attempts to relieve the symptoms of her metabolic disturbances as this type of tumour is generally regarded refractory (35) These attempts were rather unsuccessful The temporary decrease in the high plasma levels of the growth hormone following radiotherapy cannot be distinguished from spontaneous variations with certainty Neither did steroid treatment

Table 1. *Laboratory investigations of the endocrine function of the patient*

Unless otherwise stated the determinations were made on plasma or serum. Range of normal values for this laboratory are given within parentheses

		Determined at age (months)
Electrolytes Na	145 mekv/l (138–150)	7
K	4.6 mekv/l (3.5–5.1)	7
Ca	4.5 mekv/l (4.5–5.8)	7
P	5.2 mg/100 ml (4.5–6.0)	7
Mg	2.1 mg/100 ml (1.7–2.9)	7
Alkaline phosphatases	7 Bach Bach U (5–16)	7
Protein bound iodine	7.3 µg/100 ml (3.5–7.5)	10
Cholesterol	174 mg/100 ml (50–200)	7
Triglycerides	0.5 mmol/ml (0.35–1.8)	13
Thyroid stimulating hormone	5 µU/ml (<14)	7
Luteinizing hormone	1.7 ng/ml (0.5–2)	20
Follicle stimulating hormone	2.0 ng/ml (0.5–2)	20
Cortisol (two different days)	43 µg/100 ml (6–30)	13
	57 µg/100 ml (6–30)	22
Cortisol in urine	190 µg/24 hrs (70–160)	15
Pregnanetriol in urine	0.2 mg/24 hrs (<1)	15
Adrenaline in urine	46 µg/g creatinine (10–30)	15
Noradrenaline in urine	14 µg/g creatinine (10–70)	15
Urea excretion in urine per day		
Three basal days	7.5 5.6 6.3 g	19
5th day on GH therapy	5.1 g[a]	

[a] No significant change ($p > 0.05$)

term periods. The only possibly effective therapy was insulin. During treatment with insulin the child increased in weight and height. The result of the intravenous glucose tolerance test showed that the exaggerated rate of lipolysis could be reduced in spite of the constantly high level of the plasma growth hormone. The effect of insulin may therefore be explained by its antilipolytic action. The growth in height continued after withdrawal of the insulin therapy. Since spontaneous remissions with increasing growth have been reported in some other cases (9) it is not possible to say whether the improvement observed was spontaneous or induced by the insulin.

The pathogenesis of the profound metabolic disturbances occurring in this syndrome, particularly the abnormal lipid metabolism, is not fully understood. The increased secretion of the growth hormone must be assumed to play an active role, even if this assumption seems paradoxical in some respects. The plasma level of growth hormone was thus as high as that found in active acromegaly and it did not respond to such influences which normally inhibit the release of growth hormone. This obviously excludes the possibility of a continuous stimulation of the pituitary gland by normal mechanisms alone, such as the permanent hyperactivity accompanying this syndrome. Moreover the hyperactivity of the patient gradually decreased during the observation period. The cause of the autonomous high secretion of growth hormone is unknown, but the location of the tumour in the anterior hypothalamus suggests interference with the function of the growth hormone releasing factor (reviewed by McCann & Porter (23)) with hypersecretion of this factor as a result. A more probable cause is that the tumour has invaded and destroyed certain areas of the hypothalamus. If such lesions affect the site of origin of the proposed growth hormone inhibiting factor (17), they might well cause hypersecretion of growth hormone. This syndrome may thus lend clinical support to the assumed existence of such a growth hormone inhibiting factor.

The growth hormone secreted in these cases obviously retains its lipolytic effect but loses

Table 2 *Plasma growth hormone (GH) ng/ml plasma insulin μU/ml glucose mg/100 ml free fatty acids (FFA) mmol/l and glycerol mmol/l by different types of tests*

Test		Time (minutes)										Age (months)
		−15	0	30	60	90	120	180				
Glucose 10 g p o	GH		48	41	37	21		39				
	Insulin		6		12	18		8				7
	Glucose		74	130	156	107		38				
Glucose 10 g p o after 5 weeks therapy with Dexamethasone	GH	> 50	> 50	> 50	> 50	> 50		> 50				
	Glucose	54	54	122	224	234		106				13
Insulin 0 1 U/kg i v	GH	43	49	42	17	15	45					
	Glucose	—	64	40	44	42	44					10
		Time (minutes)										
		−10	0	5	10	15	20	30	45	60	90	
Glucose 0 5 g/kg i v kG value 2 6	GH	21	18	22	25	26	31	33	29	22	13	
	Glucose	45	49	185	175	152	120	100	69	56	57	
	Insulin	4	6	10	6	5	8	16	10	10	7	24
	FFA	2 13		1 50		1 37			0 97		1 09	
	Glycerol	0 75		0 46		0 44			0 30			
		Time (hours)										
		0	2	5								
Reserpine 2 5 mg i m	GH	45	65	65								1
		Time (days)										
		0	1	2								
Chlorpromazine 15 mg p o and i m on day 2	GH	8	6	21								28
		Time (days)										
		0	2									
GH injections 2 mg i m *on 2 consecutive days*	Sulfation factor	0 70	0 42									26
	(95 conf interval)	(0 49–0 98)	(0 27–0 57)									
		Time (date)										
		8/7	16/7	23/7	31/7	7/8	-4/8					
Radiotherapy 3950 rad Date 29/6–11/8	GH	40	26	14	11	33	28					15

its growth promoting ability The loss of adipose tissue is not so striking in other conditions with severe hypersecretion of growth hormone In acromegaly and gigantism the raised insulin response might be a preventive factor As in the case reported by Beck et al (3) the insulin response was low in our patient The emaciation may therefore perhaps be due to a disturbance of the interaction between insulin and growth hormone On the other hand, when diabetes develops in acromegaly at the state of pancreatic exhaustion no emaciation occurs Furthermore in the Laron type of dwarfism (18 19) or in the group III type of ateliotic dwarfs in the classification of Merimee et al (24) both associated with high plasma levels of growth hormone the insulin *response is* low yet both diseases are characterized by obesity Thus factors other than an inbalance between growth hormone and insulin secretion must be involved The finding of relatively low fasting blood glucose concentra

Table 3 *Plasma concentration of individual amino acids*

Amino acid	Umol/l
Taurine	55
Aspartic acid	79
Threonine	135
Serine	145
Asparagine	51
Glutamic acid	145
Glutamine	634
Proline	256
Glycine	193
Citrulline	29
Alanine	313
Alfa amino butyric acid	9
Valine	201
Methionine	37
Isoleucine	80
Leucine	135
Tyrosine	89
Phenylalanine	73
Ornithine	138
Lysine	198
Histidine	110
Arginine	43

tions (also described by Russell) the high K_G value and the slow return of blood glucose to baseline after i v insulin are characteristic of lack of growth hormone rather than of hypersecretion of this hormone Hence this syndrome is characterized by an increased lipolysis but rather a decreased growth hormone effect on glucose metabolism All knowledge of the effects of growth hormone hypersecretion is based on observations in older patients Therefore it is not possibl to say if these findings are specific for this particular disease or is linked just to an early age But the diabetogenic effect of growth hormone is obviously not solely the effect of an increased lipolysis

The retardation of growth in the presence of high plasma levels of growth hormone is remarkable but occurs also in some other conditions (Table 4) It may thus be seen in severe proteincalorie malnutrition (29) However the normal content of amino acids in plasma of the actual case did not suggest that the grave emaciation was associated with any protein depletion (37)

In some respects our case resembled the Laron type of dwarfism (19) Besides a high plasma growth hormone level there was a low sulfation factor (Table 4) As Laron dwarfs do not respond to therapeutic doses of HGH their growth retardation has been ascribed to refractory receptor sites owing either to defects in the receptors or to blockage by a biologically defect growth hormone retaining its immunoreactivity (6) In our patient administration of growth hormone had no effect on growth in a long term trial and did not cause nitrogen retention in the short term test If the primary cause of the retardation of growth was the secretion of a modified growth hormone treatment with normal HGH should have induced accelerated growth Furthermore the change in this syndrome between periods of retarded and accelerated growth implies that the modified growth hormone is secreted for only a limited period followed by the production of normal hormone This also argues against the existence of a modified growth hormone

The assumption of a normal growth hormone implies a defect in the synthesis of sulfation *factor* This hypothesis fits in with the data available A similar disturbance has been proposed to occur in Laron dwarfism Whether such a defect can be induced by exhaustion of the production of the sulfation factor following prolonged hypersecretion of growth hormone *is not known* No such effect is found by growth hormone producing adenoma of the pituitary But as eosinophilic adenoma of the pituitary is extremely rare in childhood the youngest patient with gigantism and proven hypersecretion of growth hormone we know of was 12 years old (22) no definite conclusions can be drawn that are valid in this low age The reports of gigantism starting very early in life eg the Alton giant below 1 year of age (4) might weigh against the exhaustion mechanism but the lack of growth hormone data from these cases reduces the validity of such comparisons

The patient showed accelerated growth until 6 months of age despite emaciation with re

Table 4 *Different forms of retardation of growth with abnormal secretion of or response to, grow[th] hormone*

Features	Diencephalic emaciation	Dwarfs with elevated HGH	Pygmies	Protein calorie deficiency	Pituitary dwarfs
Clinical features					
Subcutaneous fat	Highly reduced	Increased	Normal	Reduced	Increased
Skeletal age	Normal or advanced	Retarded	—	—	Retarded
Dentition	Normal	Retarded	—	—	Retarded
HGH					
Basal level	Elevated	Elevated	Normal	Elevated	Low
Response to stimulation	Autonomous or increased	Normal or increased	Normal	—	No
Response to suppression	No or paradoxal	Normal or paradoxal	—	Decreased	—
Sulfation factor					
Basal level	Low	Low	Normal	Low?	Low
Increase after HGH therapy	No	No	No	—	Yes
Insulin response to stimulation	Low	Low	Low	Normal or increased	Low
FFA basal	High	Normal or increased	Normal	Normal or increased	Normal or decreased
Effect on HGH therapy on					
Nitrogen retention	No	No	No	—	Yes
Growth velocity	No	Weak	—	—	Yes

markable weight loss (Fig 3) This also lends support to our assumption of the secretion of a normal growth hormone that initially had an effect on growth in length

As only very little information is available on the requirements for the synthesis of sulfation factor other mechanisms may well be the cause of the supposed block The data collected from the case reported here show that the metabolic disturbances do not correspond to any other of the known conditions with retarded growth and abnormal growth hormone secretion The data available do not permit elucidation of the precise pathogenesis of the syndrome The special features distinguishing it from other diseases with hypersecretion of growth hormone may depend on secondary factors, such as the patient s age or on the occurrence of concomitant primary or secondary abnormalities in other hormonal or neural systems, as yet not revealed

SUMMARY

A girl with a large optic glioma and th[e] typical features of diencephalic syndrome o[f] emaciation (Russell) was followed up from ¹/[₂] to 2 ¹/ years of age She had very high level[s] of growth hormone (GH) in the plasma, whic[h] were not influenced by hyperglycaemia b[y] hypoglycaemia or by dexamethasone reserpin[e] or chlorpromazine Interference by the gliom[a] with GH releasing factor or more likely wit[h] GH inhibiting factor is suggested In spite o[f] the elevated plasma GH linear growth wa[s] markedly retarded Sulfation factor activit[y] (Somatomedin) was low with no significan[t] response to injections of HGH The pathogenesis of the profound metabolic disturbance[s] in this syndrome is not properly understood The endogenous GH obviously exerts its ful[l] adipokinetic effect whereas the synthesis of the sulfation factor (Somatomedin) must be

defective. This case illustrates a type of retardation of linear growth not seen in any other known syndrome.

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THE RENAL RESPONSE TO AN ORAL SALT AND FLUID LOAD IN CHILDREN WITH COARCTATION OF THE AORTA

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Studies in the experimental animal have convincingly demonstrated that intrarenal physical forces are of primary importance for the control of tubular sodium reabsorption (22 20 8) and thus in a broader sense for the control of sodium and fluid homeostasis The clinical implications of this new concept have so far mainly been a subject of speculation The only situation in man in which the importance of physical forces has been indirectly tested is when the extracellular fluid volume is expanded In such a case man responds in a fashion similar to experimental animals i e with natriuresis (28 4 3 11) It is generally suggested that this natriuretic response could be attributed to changes in intrarenal physical forces such as dilution of the peritubular capillary blood (19 6)

In the present study the renal response to an oral salt and fluid load was tested in a group of children with coarctation of the aorta Studies were made before and after surgical resection of the coarctation The project was initiated by a previous observation that in dog renal artery perfusion pressure was directly related to urinary sodium excretion this relationship most likely being mediated by intrarenal physical forces (5)

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MATERIAL

Six patients 1 girl and 5 boys aged 6–15 years were studied All the children were normally developed and in excellent physical condition at the time of the study None of the patients received digitalis or any other therapy One patient M H (Table 1) was for a short period during infancy in cardiac failure but had thereafter not felt any symptoms from the disease In the rest of the patients the disorder had been diagnosed at routine physical examinations at pediatric health centres or in school The diagnosis was confirmed with aortography in all patients The coarctation was of the usual adult type in all cases Two of the patients C W and M W (Table 1) had associated cardiovascular malformation patent ductus arteriosus and atrial septal defect, respectively None of the patients had a history of urinary tract infection or other renal disease Table 1 lists blood pressure in all patients from preoperative and postoperative (time of the second study) cuff measurements of right arm and intra-aortic recordings of the pressure gradients across the coarctation made immediately before resection Since the latter recordings were made under varying anaesthetic depths the absolute blood pressure values are not considered to be representative and are not included in the table When the right arm cuff recordings are compared with the standards given by Graham et al (14) 5 of the 6 patients had moderately elevated arterial pressure in the upper extremities The preoperative pulse amplitude averaged 48 ± 12 mmHg (mean ± 1 S D) Postoperatively there was a fall in the mean arterial pressure averaging 11 ± 6 mmHg (± 1 S D) Preoperatively a pressure gradient across the coarctation was a consistent finding The gradient averaged 30 ± 6 mmHg (mean ± 1 S D) Immediately following the resection of the coarctation the gradient was abolished This was accomplished by close to equal gain in distal and fall in proximal aortic pressure Before resection the pulse amplitude distal to the coarctation was damped (Fig 1) in all patients

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Table 2 *Preoperative renal functional data*

Patient	GFR (ml/1 73 m² b s /min)	C_{PAH} (ml/1 73 m b s /min)	Filtration fraction ( )	Urinary Na elimination rate (mEq/1 73 m² b s /hour)
M H	166	503	33	7 5
C K	184	563	33	8 3
H S	157	—	—	1 9
C W	143	576	25	10 0
M W	115	478	27	6 8
P G	156	519	30	9 0
Mean _S D	154 ± 23	519 ± 58	30 ± 4	7 3 ± 2 9
Controls mean ± S D	123 + 16	546 ± 47	21 ± 1	16 0 ± 1 8

5 boys and 1 girl aged 8–14 years

The results from those studies that were carried out in this laboratory at the time when the study of the coarctation patients was going on will be reported in detail elsewhere (7) The glomerular filtration rate was somewhat higher in patients with coarctation of the aorta than in the controls The PAH clearance was *not* significantly different Thus there was a significant increase in the filtration fraction in the patients with coarctation of the aorta The urinary sodium elimination rate was low in the patients with aortic coarctation and the difference from the control group was highly significant

Fig 2 demonstrates the changes in glomerular filtration rate PAH clearance filtration fraction and sodium elimination rate following resection of the coarctation In all cases there was a tendency towards normalization of the filtration fraction and the sodium elimination rate Since the glomerular filtration rate was

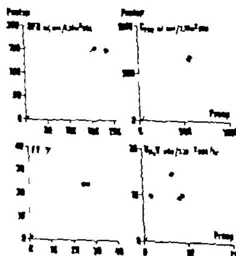


Fig The effect of resection of the coarctation on glomerular filtration rat (GFR) PAH clearance (C) filtration fraction (FF) and urinary sodium excretion after an oral salt load (U V) Pre and postoperative determinations of PAH clearance and filtration fraction in the same patient were only obtained in 4 cases.

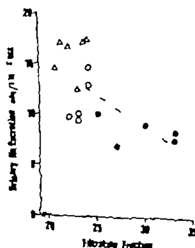


Fig 3 The relationship between filtration fraction and hourly urinary sodium excretion after an oral salt load preoperatively (●) postoperatively (○) and in controls (Δ) The correlation coefficient is −0 62 which is significant

Table 1 *Arterial pressure recordings*

Patient	Age (years)	Sex	Right arm cuff pressure (mmHg) Preoperative	Postoperative	Pressure gradient across coarctation (mmHg)
M H	6	M	140/90–112[a]	130/80–102	24
C K	7	M	140/80–106	125/75–97	35
H S	10	M	130/100–107	110/80–93	32
C W	11	F	130/90–107	105/75–88	35
M W	11	M	125/80–100	115/80–95	20
P G	15	M	150/90–116	140/80–102	35

[a] Mean pressure calculated as diastolic pressure + 43 of the pulse

studied Operation was performed in normothermia After resection the anastomosis was sutured over and over In no case was a graft used The postoperative course was uneventful in all patients

METHODS

Studies were carried out 2 to 5 days preoperatively and 7 to 10 days postoperatively The children had been hospitalized at least 3 days prior to the first study The daily sodium intake before the studies averaged 100 mEq/1 73 m body surface/day with little fluctuations

All urine samples were obtained by spontaneous voiding For blood sampling and injection purposes infusion cannulas (Stille Werner AB) were inserted into two superficial brachial veins

All studies were carried out under relatively constant degree of fluid expansion For this purpose the patients ingested water in an amount corresponding to 2-2 5 of the body weight during 1 hour and thereafter in amounts corresponding to 0 5 of the body weight every 30 minutes The first urine collection period was generally obtained 2-3 hours after the large fluid intake was started Urine was then collected at hourly intervals During the second urine collection period the oral salt load was given as tablets

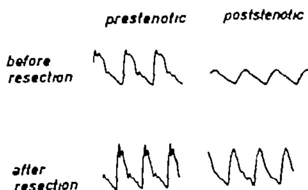


Fig 1 Intraaortic pressure recordings in patient M H at the time of operation

of NaCl in the dosage of 95 mEq Na/1 73 m² body surface The tablets were ingested during the first 15 minutes of the second urine collection period A detailed description of the oral salt load test has been given elsewhere (7) A criterion for a reliable test is that the urinary Na^+ excretion increases during the first 2 hours following the load and thereafter reaches a plateau which will last for at least 4 hours The capacity to excrete the acute load is then measured as the average urinary sodium excretion 3-6 hours following the salt intake Only those studies are used where the 2-6 hour plateau is stable i e the hourly sodium excretion does not vary more than 20

2-3 hours following the oral salt load a single i v injection of a solution containing 9 inulin (Laevosan Gesellschaft) and 18 PAH (MSD) was given in the amount of 0 75 ml/kg body weight Venous blood samples were obtained every 5 minutes during the first 20 minutes following the injection and thereafter every 10 minute during another 60 minutes From the plasma disappearance rates so obtained the clearances of inulin and PAH could be calculated using the formula given by Sapirstein (24)

Serum samples were analysed for inulin according to the method of Heyrovsky (16) and for PAH according to the method of Smith (27)

Sodium concentration in urine was determined with a flame photometer (Eppendorf)

In 5/6 patients preoperatively and in 3/6 patients postoperatively a 24 hour urine sample was obtained 1 day prior to the study and analysed for aldosterone by a modification of the double isotope method (18)

RESULTS

The average results from the preoperative studies are listed in Table 2 The values obtained have been compared with those found in a group of children of same age but without signs of circulatory or renal disturbances

rate during preserved total renal blood flow (1) Previous studies of acute variations in renal artery pulse have not revealed any effect on glomerular filtration rate or effective renal plasma flow (26) Presently it therefore seems most likely that the *chronically* damped renal artery pulse possibly in combination with a moderate reduction in mean perfusion pressure has given rise to a series of events in which the increased filtration fraction is one of the end results

The urinary sodium elimination rate in the children with aortic coarctation was found to be low and then increased postoperatively This is in agreement with the findings that in coarctation of the aorta the sodium clearance is reduced (30) and the natriuretic response following an intravenous saline load is small (21) In a previous study in children with recurrent urinary tract infections the urinary sodium elimination rate following the same salt and fluid load was found to correlate directly to the glomerular filtration rate (3) In coarctation of the aorta glomerular filtration rate is supernormal The reduced natriuretic response following the load must therefore be attributed to a reset of glomerular tubular balance for sodium with enhancement of tubular sodium reabsorption Intrarenal physical forces are generally considered to be of primary importance for the control of tubular sodium reabsorption and glomerular tubular sodium balance Intrarenal physical forces include the hydrostatic and oncotic pressure gradients between the peritubular capillaries and the peritubular interstitium adjacent to the tubular epithelial cells Low hydrostatic and high oncotic pressure in the peritubular capillaries are thought to enhance the reabsorption of sodium The renal artery perfusion pressure in the children of the present study was most likely not markedly reduced but might have been subnormal The complex influences of the damped pulse and the deviations in afferent—efferent arteriolar vascular resistance will however make the final effect on the hydrostatic pressure in peritubular capillaries unpredictable On the other hand it is most likely that the oncotic pressure in the peritubular capillaries is increased in coarctation of the aorta since the filtration fraction is increased which should lead to concentration of the plasma protein content in the postglomerular vessels A causal relationship between oncotic pressure in the peritubular capillaries and the Na reabsorption of the proximal tubules has recently been definitely established (8) In the present study the pre and postoperative as well as the control values for the urinary sodium excretion were found to correlate inversely with the filtration fraction This finding suggests that the reduced urinary sodium excretion found in patients with coarctation of the aorta could be wholly attributed to intrarenal physical changes Among other factors known to influence tubular sodium reabsorption only the level of aldosterone was evaluated and found to be normal and unchanged postoperatively

It is thus postulated that the redistribution of intrarenal vascular resistance in patients with coarctation of the aorta will result in a maintenance of the glomerular filtration rate as well as a reset of glomerular tubular balance with an enhancement of tubular sodium reabsorption The former effect is definitely an important biological adaptation the latter effect might be a pathophysiological byproduct resulting in subclinical sodium retention This byproduct might influence the pathology of the disorder It is generally agreed that the hypertension in aortic coarctation is not only due to mechanical factors (15) Experimental studies have strongly suggested that kidneys perfused from vessels originating below the coarctation are necessary to the development of the hypertension (25 29) The role of the kidney in hypertension of aortic coarctation was first thought to be linked to the renin angiotensin system There is now substantial evidence that the renin and angiotensin levels are not elevated in patients with coarctation of the aorta (2 32) Aside from the production of specific hypertensive sub-

Table 3 Twenty four hour urinary excretion of aldosterone

	µg/1 73 m² b s /24 hr mean	Range
Preoperatively	9 3	6 8–13 2
Postoperatively	9 7	6 3–12 4
Normal controls		4–17

not uniformly reduced postoperatively the reduction of the filtration fraction must be ascribed to changes in PAH clearance as well as to changes in glomerular filtration rate

Since variations in filtration fraction are thought to effect intrarenal physical forces by changes of the oncotic pressure in the peritubular capillaries the relationship between urinary sodium elimination rate and the filtration fraction has been examined in Fig 3 Values from pre and postoperative studies as well as from control studies are included The correlation coefficient obtained was statistically significant $(0.01 > p > 0.001)$

Table 3 gives the pre and postoperative 24 hour urinary excretion of aldosterone Normal values were obtained pre as well as postoperatively

DISCUSSION

For interpretation of the renal functional pattern in aortic coarctation a definition of the circulatory deviations effecting the kidney will be necessary Unfortunately no absolute data on aortic or renal artery pressure/flow relationships were obtained in the present study Intra aortic and femoral artery recordings made in other laboratories have, however clearly demonstrated that the most remarkable poststenotic change is a reduction of the pulse amplitude (9 15) A damped poststenotic aortic pulse preoperatively was also a consistent finding in our patients The mean poststenotic arterial pressure in aortic coarctation has been reported as subnormal to elevated The only situation in which the mean femoral artery pressure is remarkably low is the first weeks following the creation of an experimental coarctation (25) In the children of this study the right arm pressure was only moderately elevated as compared to the pressures generally recorded in adults (15) and the pressure gradient across the coarctation was relatively high This suggests that the actual abdominal aortic pressure was subnormal rather than slightly elevated

Renal ischemia was absent in all patients studied The glomerular filtration rate was supernormal and the effective renal plasma flow was normal Previous determinations of renal hemodynamics in patients with coarctation of the aorta have shown normal filtration rates and normal or slightly reduced renal plasma flows (15 12 13 31 17) In accordance with the present findings the filtration fraction has generally been reported to be increased in coarctation of the aorta (12 13 17) The somewhat high levels of filtration rates and PAH clearances recorded in this study might be attributed to the relatively mild manifestations of the coarctation In cases of aortic coarctation with more severe hypertension the vasoconstrictive stimulus might also effect the renal vascular bed

1–2 weeks following resection of the coarctation the filtration fraction had been normalized The preoperative disproportion between glomerular filtration rate and effective renal plasma flow in aortic coarctation must therefore be of physiological origin The relatively high filtration rate suggests a dilation of the afferent arterioles Since the high filtration rate was not accompanied by any rise in effective renal plasma flow the dilation of the preglomerular arterioles must also be associated with a relative increase in the tonus of the efferent arterioles The mechanism behind this renal vascular response is obscure It was concluded that in the present patients the coarctation had resulted in a damped pulse with a mildly reduced mean pressure In acute animal experiments a fall in mean renal artery perfusion pressure from 100 to 70 mmHg has been described to result in a fall in filtration

rate during preserved total renal blood flow (1). Previous studies of acute variations in renal artery pulse have not revealed any effect on glomerular filtration rate or effective renal plasma flow (26). Presently it therefore seems most likely that the *chronically* damped renal artery pulse, possibly in combination with a moderate reduction in mean perfusion pressure, has given rise to a series of events in which the increased filtration fraction is one of the end results.

The urinary sodium elimination rate in the children with aortic coarctation was found to be low and then increased postoperatively. This is in agreement with the findings that in coarctation of the aorta the sodium clearance is reduced (30) and the natriuretic response following an intravenous saline load is small (21). In a previous study in children with recurrent urinary tract infections the urinary sodium elimination rate following the same salt and fluid load was found to correlate directly to the glomerular filtration rate (3). In coarctation of the aorta glomerular filtration rate is supernormal. The reduced natriuretic response following the load must therefore be attributed to a reset of glomerular tubular balance for sodium with enhancement of tubular sodium reabsorption. Intrarenal physical forces are generally considered to be of primary importance for the control of tubular sodium reabsorption and glomerular tubular sodium balance. Intrarenal physical forces include the hydrostatic and oncotic pressure gradients between the peritubular capillaries and the peritubular interstitium adjacent to the tubular epithelial cells. Low hydrostatic and high oncotic pressure in the peritubular capillaries are thought to enhance the reabsorption of sodium. The renal artery perfusion pressure in the children of the present study was most likely not markedly reduced but might have been subnormal. The complex influences of the damped pulse and the deviations in afferent—efferent arteriolar vascular resistance will however make the final effect on the hydrostatic pressure in peritubular capillaries unpredictable. On the other hand it is most likely that the oncotic pressure in the peritubular capillaries is increased in coarctation of the aorta, since the filtration fraction is increased, which should lead to concentration of the plasma protein content in the postglomerular vessels. A causal relationship between oncotic pressure in the peritubular capillaries and the Na reabsorption of the proximal tubules has recently been definitely established (8). In the present study the pre- and postoperative as well as the control values for the urinary sodium excretion were found to correlate inversely with the filtration fraction. This finding suggests that the reduced urinary sodium excretion found in patients with coarctation of the aorta could be wholly attributed to intrarenal physical changes. Among other factors known to influence tubular sodium reabsorption only the level of aldosterone was evaluated and found to be normal and unchanged postoperatively.

It is thus postulated that the redistribution of intrarenal vascular resistance in patients with coarctation of the aorta will result in a maintenance of the glomerular filtration rate as well as a *reset* of glomerular tubular balance with an enhancement of tubular sodium reabsorption. The former effect is definitely an important biological adaptation, the latter effect might be a pathophysiological byproduct resulting in subclinical sodium retention. This byproduct might influence the pathology of the disorder. It is generally agreed that the hypertension in aortic coarctation is not only due to mechanical factors (15). Experimental studies have strongly suggested that kidneys perfused from vessels originating below the coarctation are necessary to the development of the hypertension (25, 29). The role of the kidney in hypertension of aortic coarctation was first thought to be linked to the renin angiotensin system. There is now substantial evidence that the renin and angiotensin levels are not elevated in patients with coarctation of the aorta (2, 32). Aside from the production of specific hypertensive sub-

stances such as renin the kidney also participates in the regulation of arterial blood pressure by its control of sodium homeostasis Positive sodium balance is reported to predispose to renal hypertension (23 10) It is thus suggested that the tendency to subclinical sodium retention found in children with aortic coarctation will contribute to the development of the prestenotic hypertension

SUMMARY

The natriuretic effect of an oral salt load has been tested in 6 children before and after resection of coarctation of the aorta Pre and postoperative determinations of GFR and PAH clearances were also made Preoperatively the natriuresis following the salt load was depressed, indicating enhanced tubular sodium reabsorption The GFR was slightly elevated the PAH clearance was normal and the filtration fraction was elevated Postoperatively there was an increase in the natriuretic response and a fall in the filtration fraction There was a significant inverse correlation between the filtration fraction and the urinary excretion of the salt load The results suggest that coarctation of the aorta is associated with a shift in intrarenal vascular resistance and that this hemodynamic change will by rise in the oncotic pressure of the peritubular capillaries result in the enhancement of the tubular sodium reabsorption

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SIMULTANEOUS DETERMINATION OF PLASMA VOLUME AND TRANSCAPILLARY ESCAPE RATE WITH ^{131}I LABELLED ALBUMIN AND T 1824 IN THE NEWBORN

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Blood and plasma volume in the human neonate have been investigated by several authors using either T 1824 or ^{131}I human serum albumin (RIHSA) as indicators (6 9 16 18) Since Williams & Fine (19) introduced a semiautomatic measurement of blood and plasma volume with RIHSA it has been increasingly employed in infants The method is easy rapid and requires only 2 ml of blood Thus it has several advantages compared with the conventional T 1824 method that requires 6–8 ml of blood for dye analyses The use of radioactive substances constitutes the only disadvantage

Since the tracer loss during mixing time is high and individually highly variable in the newborns compared with the adults a considerable overestimation of plasma volume will be introduced by taking only one plasma sample as is usually done

The purpose of the present study was to investigate a new micro method for plasma volume determination with T 1824 which permits frequent plasma sampling after the injection This was made possible by use of a two wavelength spectrophotometric method which requires only 150 μl plasma for dye analyses Plasma volume determined with RIHSA was used as reference volume Plasma volumes were calculated by retropolation to zero time of the plasma disappearance curve thus eliminating the errors introduced by tracer loss during the mixing period

In addition the overall capillary permeability to albumin was determined by measuring the plasma disappearance rate during the first hour after the intravenous injection of the tracers

MATERIAL

To obtain an homogeneous group treated identically during labour and in the postnatal period 10 otherwise healthy infants born of diabetic mothers were selected for the study The infants were delivered by Caesarean section between the 34th and the 40th week of gestation and weighed 2 900–4 400 g at birth Normal Apgar score was present in all infants In order to avoid considerable plasma volume changes and consequently a non steady state during the investigation the umbilical cord was clamped early within 5 sec after presentation of the buttocks and usually prior to the first cry The investigation was carried out within the first 48 hours and in most cases within 24 hours after delivery None of the infants received any food from the time of delivery to the time of investigation

METHODS

I human serum albumin

I human serum albumin code MIMS (Institute for Atomic Energy Kjeller Norway) was used since the tracer by metabolic studies has been demonstrated to be virtually non-denaturated (15) 0 06 μCi/kg were injected at each investigation giving a total radiation dose of less than 5 mrem Thyroid uptake of radio-

active iodide was blocked by giving an oral dose of 2 drops of Lugol's solution prior to examination and 2 drops on the following day. After precipitation with 10% trichloroacetic acid the amount of free ^{125}I in the supernatant in no case exceeded 1%. The radioactivity was measured in a well type scintillation detector (Selektronik). At least 5 000 counts were recorded (S D 3). Standards were prepared with about 30 mg inactive albumin per ml in order to avoid absorption to the glass (13).

T 1824

T 1824 (Warner-Chilcott, Morris Plains, N J) was injected in doses of 0.3 mg/kg. The extinction of the samples (150 µl plasma diluted with 450 µl distilled water) was determined at 620 and 740 nm in a Zeiss spectrophotometer Model M4 QIII. Samples showing macroscopic hemolysis were discarded.

Procedure

Using sterile technique the umbilical vein was cannulated for 6 to 8 cm with a No 5 French polyethylene infant feeding tube filled with saline. After the injection of T 1824 and RIHSA into the circulation the injection syringes were flushed several times with the blood of the infant. Finally the tube was flushed with 2 ml saline and withdrawn. A new tube was then inserted in the umbilical vein and 1 1 / ml of blood was withdrawn and transferred to heparinized test tube at 10, 20, 30, 50 and 60 minutes after injection.

Residual activity in the catheter from foregoing samples was avoided by withdrawing 2 ml blood prior to each renewed sampling. This sample was reinjected after the test samples had been obtained.

Chemical analyses

In each sample hematocrit was determined by means of a 3 min centrifugation at 12 000 rpm and total protein concentration was read refractometrically with a TS meter (American Optical Comp.). Serum albumin was measured according to Laurell (8).

Calculations

In order to assess the actual blank value at 620 nm in T 1824-dyed plasma samples, the extinction of non dyed plasma samples was determined at 620 and 740 nm according to Nielsen & Nielsen (10). A total of 34 plasma samples were examined in duplicate. Blank values of each dyed sample at 620 nm were then obtained by interpolation in the nomogram (Fig 1) from the dyed plasma extinction at 740 nm.

Radioactivity and extinction were expressed as cpm/g total protein and extinction/g total protein in order to secure the same concentration level in the different samples taken from each subject. After logarithmic transformation of these ratios the regression equation for the time-concentration curve was compiled by computer.

The transcapillary escape rate TER (per cent per hour) was calculated from the slope of the regression line.

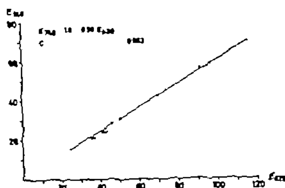


Fig 1 Correlation between extinction of non dyed plasma at 620 and 740 nm.

The outflux of albumin J (mg/kg/hour) was calculated from the *intravascular mass of albumin* IVM (g/kg) multiplied by TER.

Plasma volume PV (ml/kg) was calculated from the radioactivity and extinction at zero time by retropolation of the time-concentration curves and from the injected volume of RIHSA and T 1824 measured by weighing the syringes before and after filling them with tracer. Significance testing was carried out as a rank test for paired observations according to Wilcoxon.

RESULTS

Table 1 shows the weight and gestational age at time of investigation. Mean distribution volumes of RIHSA and T 1824 were identical being 43 ml/kg ($p > 0.1$). Consequently the intravascular mass of albumin measured by each method was identical too. A slight decrease in hematocrit and total protein concentration was recorded during the investigation due to the blood sampling ($p > 0.1$). The transcapillary escape rate and outflux of albumin is illustrated in Table 2. The difference between TER and J determined with RIHSA and T 1824 is not statistically significant ($p > 0.1$). It may be noted that the two infants (nos 7, 9) investigated only 1 hour after delivery showed the highest values for TER and J.

DISCUSSION

Plasma volume

The conventional method for plasma volume determination with T 1824 requires the use of

Table 1 *Plasma volume (PV) and intravascular mass of albumin (IVM) in newborn infants*

Subj no	Age (hour)	Weight (g)	Gestation (weeks)	RIHSA PV (ml/kg)	T 1824 PV (ml/kg)	Serum albumin (g/l)	RIHSA IVM (g/kg)	T 1824 IVM (g/kg)	Hct_{10}[a] ( )	Hct_{60}[a] ( )	TP_{10}[a] (g)	TP_{60}[a] (g)
1	20	3 600	36	41 0	41 0	30 3	1 24	1 24	50	49	4 3	4 2
2	2	3 600	36	40 5	41 5	30 2	1 22	1 25	43	43	4 4	4 2
3	15	4 200	40	36 0	34 0	34 7	1 25	1 18	58	58	5 6	5 4
4	27	3 700	36	48 0	49 0	32 1	1 53	1 57	52	51	4 8	4 6
5	5	2 900	38	44 0	47 0	33 3	1 47	1 56	49	49	4 8	4 7
6	22	3 200	36	49 5	50 5	29 5	1 46	1 49	48	48	4 5	4 5
7	1	2 950	34	33 5	33 0	35 7	1 18	1 18	62	62	5 0	5 0
8	28	4 400	37	43 5	42 5	27 6	1 20	1 17	59	59	4 7	4 5
9	1	3 000	38	53 5	52 5	36 6	1 95	1 92	38	37	4 4	4 4
10	48	3 000	37	44 5	42 0	36 6	1 63	1 53	46	45	4 1	3 9
Mean	16 8	3 455	36 7	43 3	43 3	32 7	1 41	1 41	50 4	50 0	4 6	4 5
±S D	15 3	537	1 6	6 0	6 5	3 2	0 25	0 25	7 4	7 7	0 4	0 4

[a] Hematocrit and total protein concentration 10 and 60 min after the injection of the tracers

an extraction procedure, in order to avoid the disturbing influence of the blank extinction at 620 nm The use of extraction methods is, however, time consuming and introduces a number of errors The large amount of blood required (6–8 ml) constitutes a special disadvantage in the neonate These problems were solved in the present study by using the method described by Nielsen & Nielsen (10) for calculation of the actual blank extinction at 620 nm in T 1824 dyed plasma However the greatest advantage of this method is that dye loss during the mixing time can be calculated and corrected for by taking several samples during the first hour The importance of this is stressed by the present finding of a 2–5% albumin loss during the 10 min mixing period, and by a previous investigation by Bratteby (3) showing the albumin loss amounted to 2–10% during the first 10 min after injection of the tracer

The finding in the present investigation of identical distribution volume with RIHSA and T 1824 confirms the applicability of the above mentioned method Previous investigations in adults support the present finding (1) though complete agreement was not found in all studies (21) which theoretically should be expected

Table 2 *Transcapillary escape rate (TER) and out flux of albumin (J) in newborn infants*

Subj no	RIHSA TER (/hour)	r[a]	T 1824 TER (/hour)	r[a]	RIHSA J (mg/kg/hour)	T 1824 J (mg/kg/hour)
1	13 0	−0 934	17 1	−0 965	161	212
2	15 7	−0 872	24 9	−0 979	191	311
3	14 0	−0 859	24 0	−0 932	175	283
4	15 2	−0 930	13 9	−0 941	232	218
5	17 9	−0 997	19 6	−0 994	263	306
6	13 5	−0 967	16 5	−0 906	197	245
7	25 7	−0 992	26 0	−0 969	303	307
8	18 2	−0 950	18 5	−0 992	218	217
9	34 9	−0 938	29 5	−0 906	681	567
10	16 0	−0 854	17 0	−0 904	261	260
Mean	18 4		20 7		268	292
±S D	6 8		5 1		155	104

[a] Correlation coefficient for the time-concentration curve

as both indicators are bound to albumin Jegier et al (6) investigated the blood and plasma volume in the neonate and found significantly higher values with RIHSA than with T 1824 The plasma disappearance rate of RIHSA was also significantly higher than with T 1824 These findings are probably due to the use of a partly denatured albumin preparation which according to Freeman (4) will rapidly be removed from the circulation by the liver thus resulting in too high values for plasma disappearance rate and plasma volume

Transcapillary escape rate of albumin

All infants in the present study were considered to be in a steady state with respect to the albumin exchange between the intravascular and the extravascular compartments because only small changes in hematocrit and total protein concentration occurred during the investigation These steady state criteria must be fulfilled in order to estimate the transcapillary escape rate The present mean value of TER was 18 4 %/hour Since Ingomar & Klebe (5) have obtained TER values practically identical with the present results in newborns of non diabetic mothers the possible influence of the diabetic state of the mothers on TER in the babies can probably be ruled out Further information on the total capillary permeability to albumin in newborn babies (age less than 24 hours) has been obtained by determination of the disappearance rate of RIHSA and T 1824 taking one sample shortly after the intravenous injection and another 2 hours later (6 9 16) These investigators found a disappearance rate of the same order of magnitude as mentioned for TER However it is difficult if not impossible to compare these results with the above mentioned values for TER, since no information concerning steady state during the investigation of the subjects was given The importance of this objection is further stressed by the finding of plasma volume changes during early infancy especially in late-clamped infants (18)

Krasnikoff (7) determined TER with RIHSA in non fasting newborns (age 4–47 days) using the same method and criteria of steady state as in the present investigation and found a mean TER of 27 %/hour This finding is nearly 50% higher than the present mean value for TER of 18 4 %/hour The discrepancy may be explained by the fasting state of the present subjects which minimizes the splanchnic blood flow and reduces the total surface of the splanchnic capillaries This explanation is further supported by the finding of increased protein flux via the thoracic duct in dogs fed 100 ml of cream (11)

The values of transcapillary escape rate and outflux of albumin in newborns are about 3 to 4 times greater than those obtained in adults using the same method (12) This difference can be explained either by an increase in the surface area across which exchange takes place or by an increased capillary permeability or both

Since an increase in the blood flow to an organ is accompanied by an apparent increase in the surface area across which exchange takes place an apparent recruitment of capillary bed this might explain the present findings of an increased TER and J in newborns (14 20) However studies on the transcapillary exchange of high molecular substances such as inulin (mol wt 5 500) and different dextrans (mol wt 10 000–80 000) showed the exchange to be flow independent (2 17)

The difference in TER and J between adults and newborns may therefore be interpreted as evidence of increased capillary permeability to albumin (flux per unit surface area) in newborns In addition the relatively large liver in newborns may contribute significantly to the present findings due to the high permeability of the liver capillaries

The finding in the present study of a slightly higher TER with T 1824 than with RIHSA is in agreement with previous investigations in adults (12) The most likely explanation of this finding is phagocytosis of free diffusible T 1824 in the liver

SUMMARY

A new micro method for plasma volume determination with T 1824 was investigated in 10 newborn infants Dye concentration was measured with a two wavelength spectrophotometric method The method was found to be easy rapid to perform and requires only 150 μl plasma Thus several plasma samples could be obtained and the dye loss during mixing time calculated and corrected for This is of special importance in the newborn due to the high transcapillary albumin loss mentioned below Plasma volume was determined with ^{131}I human serum albumin (RIHSA) serving as reference Identical results were obtained mean 43 ml/kg Consequently it is concluded that the dye method being a non radioactive method should be preferred for plasma volume determination in the newborn Transcapillary escape rate of albumin (fraction of intravascular mass of albumin escaping to the extravascular space per unit time) was determined from the disappearance of intravenously injected RIHSA and T 1824 during the first hour after the injection The mean transcapillary escape rate with RIHSA was 18 4 / hour with T 1824 20 7 /hour ($p < 0\ 1$) These values are 3 to 4 times higher than those obtained in adults thus indicating a high capillary permeability to albumin in newborns

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EXCRETION OF BILE ACIDS IN EXTRAHEPATIC BILIARY ATRESIA AND INTRAHEPATIC CHOLESTASIS OF INFANCY

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Cholestatic jaundice in infancy is mainly due either to extrahepatic biliary atresia or to intrahepatic cholestasis of infancy ("neonatal hepatitis syndrome"). Differential diagnosis between these conditions may often be difficult in the early stages of cholestasis (5, 27). Practically all available laboratory techniques fail to provide adequate diagnostic information.

In cholestatic jaundice of infancy conjugated bilirubin and the bile acids in serum are increased (24). The increase in the serum concentrations of these compounds varies greatly in extrahepatic biliary atresia (BA) and in intrahepatic cholestasis of infancy (IHC). For this reason their quantitative estimations are of little value in assessing the severity of the disturbance of the bile acid excretion to the intestine. For further studies of this point cholic acid 24-^{14}C was intramuscularly injected and the excretion patterns of the isotope in the urine and faeces were determined (18, 21). Previous studies have shown that the isotope is excreted in urine in BA (18). In 4 patients with IHC the excretory pattern of the isotope was virtually identical with that observed in BA (21). This suggested that a severe disturbance of the bile acid excretion co-existed with intrahepatic cholestasis in those cases.

It emerges from the above observations that a virtually identical excretion pattern of cholic acid 24-^{14}C appears to be associated with BA and IHC. This prompted the investigation of the relationship between these two conditions and the synthesis of cholic and chenodeoxycholic acids by quantitative determination of the excretion of these acids in the urine. The investigation was extended to include comparative studies of BA and IHC with reference to the excretion pattern of the isotope, the nature of the labelled urinary bile acids, the turnover rate and the pool size of cholic acid after the intramuscular injection of cholic acid 24-^{14}C. These parameters were studied during the early stages of cholestasis in 5 infants with BA and 19 infants with IHC. Four of these 5 infants with BA and 2 infants of the 19 infants with IHC who developed persistent jaundice and progressive liver cirrhosis were re-examined 10–25 weeks after the first examination.

The following systematic names are given to bile acids referred to by trivial names: cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid.

CASE MATERIAL

This series included 24 patients, 16 boys and 8 girls, who were admitted to hospital with the diagnosis of obstructive jaundice. Six of these patients with progressive liver cirrhosis were re-examined 10–25 weeks after the first admission. Table 1 summarizes

SUMMARY

A new micro method for plasma volume determination with T 1824 was investigated in 10 newborn infants Dye concentration was measured with a two wavelength spectrophotometric method The method was found to be easy rapid to perform and requires only 150 μl plasma Thus several plasma samples could be obtained and the dye loss during mixing time calculated and corrected for This is of special importance in the newborn due to the high transcapillary albumin loss mentioned below Plasma volume was determined with ^{131}I human serum albumin (RIHSA) serving as reference Identical results were obtained mean 43 ml/kg Consequently it is concluded that the dye method, being a non radioactive method should be preferred for plasma volume determination in the newborn Transcapillary escape rate of albumin (fraction of intravascular mass of albumin escaping to the extravascular space per unit time) was determined from the disappearance of intravenously injected RIHSA and T 1824 during the first hour after the injection The mean transcapillary escape rate with RIHSA was 18 4 %/hour with T 1824 20 7 %/hour ($p < 0.1$) These values are 3 to 4 times higher than those obtained in adults thus indicating a high capillary permeability to albumin in newborns

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Table 2 *Standard liver function tests in the infants at the time of the first examination*

Patient	S-bilirubin Total (mg/100 ml)	S-bilirubin Conjugated ( )	S-transaminase activity GOT units/ml	S-transaminase activity GPT units/ml	S-alkaline phosphatase (units/100 ml)	S-leucineamino-peptidase (units ml)	S-gammaglutamyl transpeptidase (units/100 ml)
A							
L	11	82	165	132	—	540	2 990
J	13	92	252	313	26	350	1 480
E	8	88	126	95	—	650	3 400
S	8	94	360	234	18	440	1 000
A	8	81	116	63	28	465	2 680
IHC[b]							
J	10	70	119	75	21	510	1 800
B	9	89	50	24	17	—	—
W	8	98	110	90	15	272	790
I	14	50	103	50	11	285	192
I	7	86	1 0	108	—	—	—
K	6	83	72	44	23	490	884
I	7	86	118	134	26	180	880
F H	18	61	64	36	—	255	1 350
J R	15	67	85	70	13	815	3 400
M T	14	89	140	130	39	700	4 000
M C	6	90	74	43	?4	216	580
J C	12	100	57	51	24	197	63
C R	8	87	138	186	38	170	144
C B	3	67	118	112	35	—	—
O N	10	90	155	80	—	410	1 500
M W	11	55	400	140	—	235	573
M P	5	+	133	118	25	—	370
K N	13	97	38	49	—	270	190
C N	7	+	45	16	—	—	—
Normal values (upper limits) (19 20 21 23)	1 2	10	57	58	15	255	300

Extrahepatic biliary atresia [b] Intrahepatic cholestasis of infancy Direct reaction positive

toxoplasmosis and lues and maternal iso-immunization. In none of the patients hepatitis associated (Au)-antigen or antibodies to Au antigen were detected. The patients with significant bacteriuria were treated with antibiotics. However, the treatment had no immediate effect on the jaundice.

METHODS

Cholic acid 24 C was obtained from New England Nuclear Corp Boston Mass. The purity was checked by thin layer chromatography (TLC) followed by autoradiography. If impurities were detected the labelled cholic acid was purified by reversed phase partition chromatography on columns of hydrophobic Hyflo-Super Cel with phase system C 1 (17). The labelled cholic acid was stored as sodium salt dissolved in 80 ‰ ethanol in the refrigerator.

Aliquots of the solution of sodium cholate 24- C were transferred to ampoules. The solvent was evaporated and the ampoules sterilized and sealed. The bile salt was dissolved in 1 ml saline and injected intramuscularly. The administered dose was corrected for residual radioactivity in the syringe needle and ampoule. The amounts of isotopic sodium cholate given to the patients ranged from 0 02 to 0 05 mg (1 7.5 μCi).

Urine and faeces were collected daily for 4 days after the injection of labelled cholate. All patients wore urinary bags and paper diapers during the sampling period. As some urine was usually passed into the diapers the latter were divided into two groups: diapers containing only urine and diapers containing both urine and faeces. After each micturition the specimen of urine was stored in a refrigerator. As soon as the 24 hour collection was completed the total quantity of urine was frozen.

Extraction of urine

Urine was acidified to pH 1 with hydrochloric acid and extracted with ethyl acetate and thereafter with n-butanol. Unconjugated bile acids and glycocholic acid were extracted with ethyl acetate and taurine conjugates with butanol. The total amount of isotope in the pure urine was taken to be the sum of the isotope in the ethyl acetate and butanol extracts.

Table 1 *Clinical and laboratory findings in the 24 patients*

Patient	Sex	Onset and clearing of jaundice (weeks)	S alfa antitrypsin mg/100 ml (age weeks): During period of jaundice	S alfa antitrypsin mg/100 ml (age weeks): After clearing of jaundice	Cytomegalovirus: Isolation in urine	Cytomegalovirus: S-complement fixation antibodies (age weeks)	Bacterial growth in urine (> 10⁵ bacteria per ml) (age weeks)	Remarks
BA[b]								
H L	♂	< 2/—	522 (8)		neg	1/20 (26)	neg	Died aged 8 5 months
K J	♀	< 2/—	594 (8)		neg	1/2 (13)	pos (12 29)	Died aged 9 months
C E	♀	< 2/—	756 (36)		n d[a]	1/10 (12)	neg	Died aged 9 months
J S	o	< 2/—	372 (28)		n d	n d	neg	Died aged 11 months
M Å	♂	< 2/—	215 (8)		neg	neg (11)	neg	Polydactyly retained testis, died aged 3 months
IHC[c]								
R J	♂	< 2/—	114 (23)		neg	< 1/5 (21)	neg	Died aged 8 months
L B	♂	10/—	756 (36)		neg	< 1/5 (20)	neg	Aortic + mitral stenosis died aged 2 years 6 months
K W	♀	< 2/7	207 (6)	168 (19)	pos	1/32 (18)	neg	Neonatal thrombocytopenia
S J	♂	< 2/12		312 (95)	pos	< 1/5 (4)	neg	Neonatal thrombocytopenia
I J	♀	6/20		192 (31)	neg	< 1/5 (48)	neg	VSD + pulmonal stenosis
J K	♂	< 2/12	60 (8)	74 (20)	neg	1/5 (20)	pos (2)	
J J	♂	< 2/12		270 (31)	neg	< 1/10 (34)	neg	Down's syndrome (trisomia) died aged 2 years 5 months of malignant lymphoma
F H	♂	< 2/6		156 (52)	neg	neg (52)	pos (2)	
J R	♀	< 2/6		234 (13)	pos	1/40 (6)	pos (1)	Inclusion bodies in urine neonatal thrombocytopenia
M T	♂	< 2/16	60 (8)	66 (47)	neg	< 1/5 (18)	neg	
M C	♂	< 2/18	60 (10)	4? (28)	neg	1/4 (12)	neg	
J C	♂	< 2/7	360 (4)	222 (21)	neg	1/5 (20)	pos (2)	
C R	♀	< 2/8		276 (48)	neg	< 1/5 (17)	n d	
M W	♂	< 2/7	282 (6)	408 (56)	neg	< 1/5 (6)	n d	
C B	♀	10/16		306 (64)	neg	1/40 (65)	neg	
O N	o	< 2/12	154 (6)	92 (82)	neg	neg (6)	neg	
M P	♂	< 2/12	n d		neg	1/16 (7)	neg	
K N	o	< 2/10	252 (4)		neg	n d	neg	Coarctation of aorta
C N	♀	< 2/14	76 (7)	96 (72)	neg	1/256 (1) 1/20 (28)	pos (72)	Inclusion bodies in urine Maternal CMV titer 1/128

[a] Not determined [b] Extrahepatic biliary atresia [c] Intrahepatic cholestasis of infancy

the sex and age at onset and disappearance of jaundice the results of urine analysis for CMV and bacteria and the serum concentrations of alfa antitrypsin and complement fixation antibodies to CMV Table 2 gives the results of the conventional laboratory tests of liver function carried out at the time of the first examination

Extrahepatic biliary atresia

Explorative laparotomy was done on 5 patients (HL KJ CE JS and MA) revealing extrahepatic biliary atresia (BA) the diagnosis being confirmed at the post mortem examination of the patients

Intrahepatic cholestasis of infancy

The remaining 19 patients were grouped together under the heading of intrahepatic cholestasis of infancy (IHC) Explorative laparotomy was performed on three of these patients (LB RJ and IJ) and revealed normal bile ducts In two of the latter cases (LB and RJ) jaundice persisted and the patients died of liver failure at an early age The post mortem examination confirmed the presence of normal bile ducts In the other 17 patients jaundice subsided Twelve of these 17 patients were re examined after clearing of jaundice and the results of those studies are reported in another paper (20)

Three of the patients (KW SJ and JR) had congenital cytomegalic inclusion disease (Table 1) and 4 patients (JK MT MC and CN) had very low serum levels of alfa antitrypsin (normal values in adults in our laboratory are 267 ± 84 mg 100 ml ($M \pm 2$ SD)) In these 4 patients the low serum concentration of alfa antitrypsin persisted or even decreased (patient MC) after jaundice had disappeared (Table 1)

The following diagnoses were excluded by standard laboratory tests hereditary tyrosinemia galactosemia cystic fibrosis infectious diseases such as coxsackie B herpes simplex rubella mononucleosis listeriosis

Table 3 *Bile acid excretion in the infants at the first examination of the patients*

Patient	Age at examination weeks	Body weight kg	Creatinine in urine mg per 24 hours	Isotope excretion of administered ¹⁴C: Urine (Diapers)	Isotope excretion: Faeces	Isotope excretion: Total (Diapers contain urine and faeces)	Urinary labelled metabolites extractable with ethyl acetate (glycine conjugated) %	Urinary excretion per 24 hours: C µmol	CD µmol	C+CD µmol	Ratio C/CD	C+CD (µmol) / Bodyweight (kg)	C+CD (µmol) / Creatinine (mg)
EBA[a]													
H L	8	4.2	44	36 (4)	2	38 (0)	19	4.1	12.2	16.3	0.3	3.88	0.37
K J	8	3.8	34	(21)	3	88 (8)	59	6.7	6.6	13.3	1.0	3.50	0.39
C B	11	4.0	28	55 (11)	<1	62 (6)	54	6.6	8.7	15.4	0.8	3.85	0.55
I S	19	4.5	88	67 (4)	1	68 (0)	70	16.2	8.2	24.4	2.0	5.42	0.8
M A	8	4.0	45	57 (7)	<1	59 (<1)	40	1.6	11.0	23.6	1.1	5.90	0.52
Mean							*43*	*9.3*	*9.5*	*18.6*	*1.0*	*4.51*	*0.42*
IHC[b]													
R J	3	3.5	38	56 (9)	<1	58 (7)	41	10.0	11.7	17	0.9	6.20	0.57
L B	15	4.7	43	39 ( )	2	41 (0)	57	7.7	6.0	13.7	1.3	2.91	0.32
K W	2	0	8	67 (22)	<1	74 (12)	34	4.5	2.9	7.4	1.6	3.70	0.93
S J	2	2.9	25	75 (17)	<1	84 (9)	45	61.3	11.3	72.6	5.4	25.03	2.90
I J	10	3.3	10	96 (5)	<1	97 (<1)	64	11.7	7.4	19.1	1.6	5.79	1.91
J K	2	2.5	18	48 (14)	1	66 (18)	48	4.1	2.7	6.8	1.5	2.72	0.38
J J	4	2.6	33	82 (2)	2	91 (6)	6	8.6	8.0	16.6	1.1	6.38	0.50
F H	3	2.5	19	72 (9)	2	80 (5)	31	10.9	4.8	15.7	2.3	6.8	0.83
I R	2	2.4	9	50 (19)	3	68 (14)	63	11.6	1.7	13.3	6.9	5.54	1.48
V T	8	4.1	42	31 (3)	3	34 (0)	19	6.1	10.9	17.0	0.6	4.15	0.40
M C	9	4.7	50	56 (<1)	3	60 (0)	12	5.9	10.0	16.0	0.6	3.40	0.32
J C	4	2.8	20	64 (19)	4	69 (1)	38	18.6	5.8	24.4	3.2	8.71	1.22
C R	4	3.3	12	33 (19)	9	53 (11)	17	1.3	4.6	5.8	0.3	1.76	0.48
C B	12	3.6	2	29 (6)	10	57 (19)	30	2.5	4.6	7.1	0.5	1.97	0.32
O N	6	3.5	31	43 (<1)	10	59 (5)	83	5.4	1.4	6.8	3.9	1.94	0.22
M W	4	3.1	36	81 (<1)	13	94 (0)	66	8.9	4.1	13.0	2.1	4.19	0.36
M P	9	4.4	73	48 (0)	14	82 (0)	72	1.8	14.5	16.3	0.1	3.70	0.22
L N	4	4	27	25 (1)	24	73 (25)	89	5.0	5.0	10.0	1.0	4.17	0.37
C N	7	3.5	32	30 (2)	26	69 (13)	97	6.1	13.5	19.6	0.5	5.60	0.61
Mean							*49*	*10.1*	*6.9*	*17.0*	*1.9*	*5.48*	*0.75*

[a] Extrahepatic biliary atresia [b] Intrahepatic cholestasis of infancy

Extraction of faeces

The faeces and the diapers containing only faeces were homogenized in water in an Ultra Turrax homogenizer (Janke and Kunkel K G Staufen Germany) Four parts of ethanol were added and the mixture refluxed for 6 hours The residue was extracted for 24 hours with boiling chloroform/methanol (2 1) and thereafter with boiling chloroform for 1 hour The total amount of isotope present in the faeces was taken to be the sum of the isotope in the ethanol chloroform methanol and chloroform extracts

Extraction of diapers

Diapers containing only urine were refluxed twice for 3 hours with 80% aqueous ethanol The total urinary isotope was calculated as the sum of the isotope in these extracts and the isotope recovered in pure urine as described above The diapers containing both urine and faeces were extracted by the same technique used for the extraction of faeces

In order to determine the total isotope excretion the isotope in extracts from the diapers containing both urine and faeces was added to the total urinary and faecal isotope

Isotope determination

Aliquots of the extracts were counted in a Packard Tri Carb liquid scintillation spectrometer

Quantitative determination of urinary excretion of cholic and chenodeoxycholic acids

Thirty ml of urine were percolated through 15 g of Amberlite XAD 2 (Rohm and Hass Co Philadelphia) in a column 1 4 × 15 cm (4) The column was eluted with 60 ml of water and thereafter with 60 ml of methanol The residue of the methanol eluate was dissolved in 2 ml of ethanol and solvolysed by addition of 2 N HCl to pH 1 and 18 ml acetone followed by incubation for 24 hours at room temperature (22) After neutralization the solvent was evaporated and the residue was hydrolysed with 2 ml of 20 KOH in ethylene glycol for 20 minutes at 220 C (9) Sixteen ml 20 NaCl solution were added to the hydrolysate and after acidification to pH 1 with hydrochloric acid the mixture was extracted with ether and thereafter with butanol The residue of the ether extract was methylated with diazomethan and subjected to gas liquid chromatography (GLC) on columns of QF 1 for determination of cholic and chenodeoxycholic acids (25)

TLC separation of labelled urinary bile acids

Aliquots of the ethyl acetate and *n* butanol extracts of urine and the methylated ether extracts of urine were used for quantitative determination of bile acids were subjected to thin layer chromatography (TLC) with the phase systems described previously (18 21) The radioactive spots were located by autoradiography

Calculation

The persisting radioactivity in the organisms was plotted versus time in a semilogarithmic diagram to determine the half life of cholic acid 24 ^{14}C ($t_{\frac{1}{2}}$) Pool size of cholic acid was calculated according to Danielsson et al (8) i e pool size = $t_{\frac{1}{2}}$/ln 2 × daily synthesis of cholic acid The daily synthesis of cholic acid was taken to be the mean daily urinary excretion of cholic acid during the four days of the isotope study in patients who excreted less than 3 of the given isotope in the faeces

Additional tests

Alfa$_1$ antitrypsin in serum was determined by radial immunodiffusion technique (Partigen® Behringwerke AG) and by the electroimmunodiffusion technique according to Laurell (12) The correlation between the results of these methods was statistically significant ($n = 150$ $r = 0\,948$ and $p < 0\,001$) In this paper only the results of the radial immunodiffusion technique are reported The determination of hepatitis associated (Au) antigen and antibodies to Au antigen were performed with immunodiffusion technique Cytomegalovirus (CMV) was isolated and complement fixation antibodies to CMV were determined in the National Bacteriological Laboratory Stockholm Sweden Serum bilirubin was estimated according to Michaelsson (15) serum transaminase activity according to Karmen (11) serum alkaline phosphatase according to Buch & Buch (6) serum gammaglutamyl transpeptidase (GT) according to Szewczuk et al (26) and serum leucinaminopeptidase (LAP) according to Rutenburg et al (23) Creatinine in the urine was determined according to Chasson et al (7)

RESULTS

Quantitative excretion of cholic and chenodeoxycholic acids in urine

The total daily excretion of cholic and chenodeoxycholic acids combined in the BA group ranged from 13 3 to 24 4 μmol the corresponding values in the IHC group ranging from 5 8 to 72 6 μmol (Table 3) These great individual variations were also observed if the daily excretion was expressed in terms of μmol cholic and chenodeoxycholic acids per mg creatinine or per kg body weight There was no difference between the two groups with regard to the ratio cholic/chenodeoxycholic acid (Table 3)

In four of the 5 patients with BA who were re examined the urinary excretion of cholic

The determinations of Au antigen and antibodies to Au antigen were kindly performed by Dr Rebekka Berg Stockholm County Council Microbiological Laboratory Stockholm

Table 3. *Bile acid excretion in the infants at the first examination of the patients*

Patient	Age at examination weeks	Body weight kg	Creatinine in urine mg per 4 hours	Isotope excretion of administered cholic acid-^{14}C: Urine (Diapers)	Isotope excretion: Faeces	Isotope excretion: Total (Diapers containing urine and faeces)	Urinary labelled metabolites extractable with ethyl acetate (glycine conjugates)	Urinary excretion per 24 hours: C µmol	CD µmol	C+CD µmol	Ratio C/CD	C+CD (µmol) / Bodyweight (kg)	C+CD (µmol) / Creatinine (mg)
BA[a]													
H L	8	4.2	44	36 (4)	2	38 (0)	19	4.1	12.2	16.3	0.3	3.88	0.37
K J	8	3.8	34	77 (21)	3	88 (8)	59	6.7	6.6	13.3	1.0	3.40	0.39
C E	11	4.0	28	55 (11)	<1	62 (6)	54	6.6	8.7	15.4	0.8	3.85	0.55
J S	19	4.5	88	67 (4)	1	68 (0)	70	16.2	8.2	24.4	2.0	5.42	0.28
M Å	8	4.0	45	57 (7)	<1	59 (<1)	40	12.6	11.0	23.6	1.1	5.90	0.52
Mean							43	*9.3*	*9.3*	*18.6*	*1.0*	*4.51*	*0.42*
IHC[b]													
R J	3	3.5	38	56 (9)	<1	58 (2)	41	10.0	11.7	21.7	0.9	6.70	0.57
L B	15	4.7	43	39 (2)	2	41 (0)	57	7.7	6.0	13.7	1.3	2.91	0.32
K W	2	2.0	8	62 (72)	<1	74 (12)	34	4.5	2.9	7.4	1.6	3.70	0.93
S J	2	2.9	25	75 (17)	<1	84 (9)	45	61.3	11.3	72.6	5.4	25.03	90
I J	10	3.3	10	96 (5)	<1	97 (<1)	64	11.7	7.4	19.1	1.6	5.79	1.91
J K	2	3	18	48 (14)	1	66 (18)	48	4.1	2.7	6.8	1.5	2.72	0.38
J J	4	1.6	33	81 (2)	2	91 (6)	26	8.6	8.0	16.6	1.1	6.38	0.50
P H	3	2.5	19	72 (9)	2	80 (5)	31	10.9	4.8	15.7	2.3	6.28	0.83
J R	2	2.4	9	50 (19)	3	68 (14)	63	11.6	1.7	13.3	6.9	5.54	1.48
M T	6	4.1	42	31 (3)	3	34 (0)	19	6.1	10.9	17.0	0.6	4.13	0.40
M C	9	4.7	50	56 (<1)	3	60 (0)	12	5.9	10.0	16.0	0.6	3.40	0.32
J C	2	2.8	0	64 (19)	4	69 (1)	38	18.6	5.8	4.4	3.2	8.71	1.22
C R	4	3.3	12	33 (19)	9	53 (11)	17	1.3	4.6	5.8	0.3	1.76	0.48
C B	17	3.6	22	29 (6)	10	57 (19)	30	2.5	4.6	7.1	0.5	1.97	0.32
O N	6	3.5	31	43 (<1)	10	59 (5)	83	5.4	1.4	6.9	3.9	1.94	0.22
M W	4	3.1	36	81 (<1)	13	94 (0)	16	8.9	4.1	13.0	2.1	4.19	0.36
M P	9	4.4	73	48 (0)	14	82 (70)	72	1.8	14.5	16.3	0.1	3.70	0.22
K N	4	2.4	27	25 (1)	24	73 (25)	89	5.0	5.0	10.0	1.0	4.17	0.37
C N	7	3.5	32	30 (7)	26	69 (13)	97	6.1	13.5	19.6	0.5	5.60	0.61
Mean							49	*10.1*	*6.9*	*17.0*	*1.9*	*5.48*	*0.75*

[a] Extrahepatic biliary atresia. [b] Intrahepatic cholestasis of infancy.

Table 4 *Clinical and laboratory findings and bile acid excretion in the infants suffering from progressive cirrhosis at the time of the re-examination*

Patient	Age in weeks at the re-examination	Body weight kg	Creatinine in urine mg per 24 hours	S bilirubin mg/100 ml (conjugated / of total)	Isotope excretion of administered cholic acid-24-^{14}C: Urine (Diapers)	Faeces	Total (Diapers containing urine and faeces)	Urinary labelled metabolites extractable with ethyl acetate (glycine conjugates)	Urinary excretion of cholic (C) and chenodeoxycholic (CD) acids per 24 hours: C µmol	CD µmol	C+CD µmol	Ratio C/CD	C+CD (µmol) / Bodyweight (kg)	C+CD (µmol) / Creatinine (mg)
BA[a]														
H L	33	7 0	30	15 (86)	55 (18)	1	67 (11)	86	6 2	9 5	15 7	0 7	2 24	0 52
K J	30	5 8	15	20 (94)	27 (14)	1	42 (13)	83	1 1	2 9	4 0	0 4	0 69	0 27
C E	35	4 3	26	22 (100)	n d[c]				3 2	3 2	6 4	1 0	1 49	0 25
J S	28	5 0	79	n d[c]	n d				4 0	10 8	14 8	0 4	2 96	0 19
IHC[b]														
R J	22	6 8	70	2 4 (92)	40 (1)	5	46 (1)	73	14 8	22 0	36 8	0 7	5 41	0 53
L B	25	5 8	61	2 2 (46)	82 (12)	<1	82 (0)	65	17 8	17 8	35 6	1 0	6 14	0 58

[a] Extrahepatic biliary atresia [b] Intrahepatic cholestasis of infancy [c] Not determined

and chenodeoxycholic acids was found to have decreased whereas it was noted to have markedly increased in the two patients with IHC associated with progressive cirrhosis (LB and RJ) (Table 4) In all but one (HL) of the re examined patients the ratio cholic to chenodeoxycholic acid was found to have decreased (Table 4)

Isotope excretion

The labelled compounds were considered to be completely recovered in the ethyl acetate and butanol extracts of urine as only trace amounts of isotope were present in the water phase after the butanol extraction Most of the labelled bile acids in the faeces were extractable with ethanol and only small amounts were found in the chloroform methanol extracts Table 3 gives the recoveries of the administered isotope in the urine faeces and diapers containing only urine and diapers containing both urine and faeces Only in 6 of the 24 patients was it possible to obtain almost complete separate samples of urine and faeces respectively In these cases less than 5% of the injected isotope was recovered in the diapers the corresponding values in several of

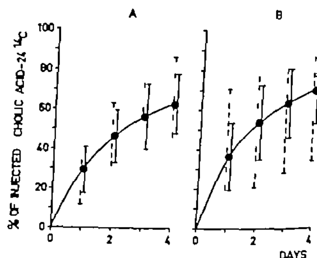


Fig 1 Cumulative excretion of total isotope in urine and faeces after intramuscular injection of cholic acid 24 ^{14}C to infants with extrahepatic biliary atresia (A) and intrahepatic cholestasis of infancy (B) at the first examination Mean (●) range ( ) and ±S D (—)

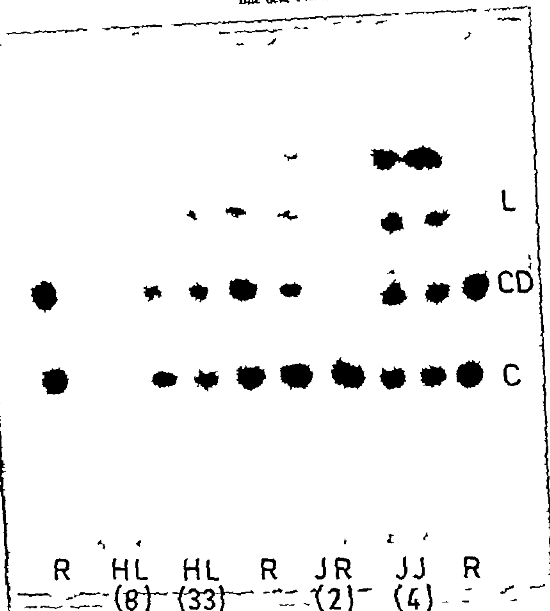


Fig. 2. Thin layer chromatogram of methylated ether extracts of urine from patients *HL*, *JR* and *JJ*. Age in weeks in parentheses. One thousandth of a 24 hour urine sample applicated on TLC plates. Reference compounds (*R*): methyl esters of lithocholic acid (*L*), chenodeoxycholic acid (CD) and cholic acid (C). Solvent system: Trimethylpentan/isopropyl alcohol/acetic acid 60:20: . Plates developed with phosphomolybdic acid.

the other 18 patients being much higher than 5.4 (Table 3).

The percentage total isotope *excretion* during the 4 day sampling period *ranged* from 34 to 97% (Table 3). There was no noteworthy difference between the BA group and the IHC group with respect to the cumulative excretion of isotope (Fig. 1). Most of the isotope was recovered in the urine (Table 3). In the BA group less than 3 of the injected isotope was recovered in the faeces. In 11 of th 19 patients in the IHC group the faecal excretion was as low as that in the BA group, i.e. less than 3%. In the remaining 8 infants 4–26%

Table 4 Clinical and laboratory findings and bile acid excretion in the infants suffering from progressive cirrhosis at the time of the re-examination

Patient	Age in weeks at the re-examination	Body weight kg	Creatinine in urine mg per 24 hours	S bilirubin mg/100 ml (conjugated % of total)	Isotope excretion of administered cholic acid-24-¹⁴C: Urine (Diapers)	Faeces	Total (Diapers containing urine and faeces)	Urinary labelled metabolites extractable with ethyl acetate (glycine conjugates)	Urinary excretion of cholic (C) and chenodeoxycholic (CD) acids per 24 hours: C µmol	CD µmol	C+CD µmol	Ratio C/CD	C+CD (µmol) / Bodyweight (kg)	C+CD (µmol) / Creatinine (mg)
BA[a]														
H L	33	7 0	30	15 (86)	55 (18)	1	67 (11)	86	6 2	9 5	15 7	0 7	2 24	0 52
K J	30	5 8	15	20 (94)	27 (14)	1	42 (13)	83	1 1	2 9	4 0	0 4	0 69	0 27
C E	35	4 3	26	22 (100)	n d[c]				3 2	3 2	6 4	1 0	1 49	0 25
J S	28	5 0	79	n d[c]	n d				4 0	10 8	14 8	0 4	2.96	0 19
IHC[b]														
R J	22	6 8	70	2 4 (92)	40 (1)	5	46 (1)	73	14 8	22 0	36 8	0 7	5 41	0 53
L B	25	5 8	61	2 2 (46)	82 (12)	<1	82 (0)	65	17 8	17 8	35 6	1 0	6 14	0 58

[a] Extrahepatic biliary atresia [b] Intrahepatic cholestasis of infancy [c] Not determined

and chenodeoxycholic acids was found to have decreased whereas it was noted to have markedly increased in the two patients with IHC associated with progressive cirrhosis (LB and RJ) (Table 4) In all but one (HL) of the re examined patients the ratio cholic to chenodeoxycholic acid was found to have decreased (Table 4)

Isotope excretion

The labelled compounds were considered to be completely recovered in the ethyl acetate and butanol extracts of urine as only trace amounts of isotope were present in the water phase after the butanol extraction Most of the labelled bile acids in the faeces were extractable with ethanol and only small amounts were found in the chloroform methanol extracts Table 3 gives the recoveries of the administered isotope in the urine faeces and diapers containing only urine and diapers containing both urine and faeces Only in 6 of the 24 patients was it possible to obtain almost complete separate samples of urine and faeces respectively In these cases less than 5% of the injected isotope was recovered in the diapers the corresponding values in several of

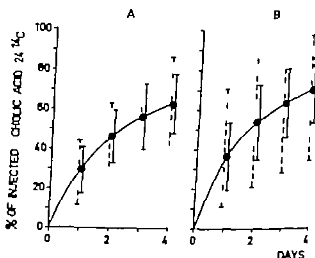


Fig 1 Cumulative excretion of total isotope in urine and faeces after intramuscular injection of cholic acid 24 ¹⁴C to infants with extrahepatic biliary atresia (A) and intrahepatic cholestasis of infancy (B) at the first examination Mean (●) range ( ) and ±S D (—)

BA and IHC groups In 2 cases of IHC associated with persisting jaundice (LB and RJ) and in 4 cases of BA (HL KJ CE and JS) the urinary excretion of these acids was found to be much higher in the former than in the latter at the re examination of the patients It is interesting to note that the ratio cholic to chenodeoxycholic acids varied greatly in both groups at the first examination of the patients However the ratio was found to be lower in 5 of the 6 patients at their re-examinations

The excretion pattern of isotope after intramuscular injection of isotopic cholic acid did not differ in the cases of BA and most of the cases of IHC However out of the 19 patients with IHC 8 patients excreted more than 3% of the injected isotope in the faeces at the first examination In these cases the excretion pattern of isotope was incompatible with a diagnosis of BA

The nature of the urinary bile acids has been previously studied in BA (18) Cholesterol-4-^{14}C was found to be mainly converted into labelled cholic and chenodeoxycholic acids which were excreted in the urine However the urine also contained small amounts of other labelled bile acids which were not products of transformation of either cholic or chenodeoxycholic acids One of these unidentified labelled bile acids was previously shown to be 3 β hydroxy 5-cholenoic acid and was excreted in large amounts in cases of BA (14) However the low incorporation of radioactivity into this acid from isotopic cholesterol suggested that cholesterol was not its direct precursor It may therefore be assumed that there is a minor metabolic pathway in bile acid synthesis in BA (2 16) All samples of urine from the patients in this series were found to contain bile acids in addition to cholic and chenodeoxycholic acids The TLC mobility and GLC retention time indicated that one of these acids was 3 β hydroxy 5 cholenoic acid which was consistently found in all samples

Conjugation of injected isotopic cholic acid did not appear to be seriously affected neither in the BA group nor in the IHC group as labelled conjugated bile acids predominated in the urine Even in the cases of BA and IHC with co-existent progressive liver cirrhosis the labelled unconjugated bile acid excretion was not increased at the re-examination of the patients

Half life and pool size of cholic acid varied greatly in both the BA and IHC group In normal infants the pool size of cholic acid has so far not been determined For this reason the relationship between cholestasis and the latter is still unknown The pool size of cholic acid per kg body weight was much smaller in the cases in this series than that which has been observed in normal adults (8 13 31) In adults with liver cirrhosis the pool size has been reported to decrease (30) There was no difference of the patients with IHC associated with congenital CMV infection and/or decreased serum concentrations of alfa$_1$ antitrypsin and the other patients with regard to the bile acid metabolism Furthermore the character of disturbance of bile acid excretion into bile was found to be identical in most cases of IHC with that in the cases of BA with total obstruction or absence of the bile ducts in this series In the cases in this series the isotope excretion pattern and the urinary excretion of total bile acids was similar in character to that observed in cases of erythroblastosis complicated by cholestasis (19) The above observations suggest that cholestasis in the newborn and infants is associated with a virtually complete suppression of the bile acid excretion to the intestine irrespective whether another disease co-exists or not On the contrary adults with liver disease and raised serum conjugated bilirubin have not been shown to have a similar severe disturbance of bile acid excretion (1 3 10 28)

Standard laboratory tests of liver function Various standard chemical tests used for study

of the injected isotope was excreted in the faeces

In 4 patients with persisting jaundice the isotope study was repeated 10–25 weeks after the first examination (Table 4) Patients with BA were found to excrete less than 1 in the faeces whereas those with IHC excreted 1–5° The rates of excretion of the isotope at the re-examination of the patients did not differ consistently from those observed at the first examination (Table 4)

Nature of urinary bile acids

TLC analysis showed that small amounts of labelled cholic acid often were present in the first 24 hour sample Trace amounts were detected in the second 24 hour sample from 4 of the 24 infants (KJ CR CB and ON) No unconjugated labelled cholic acid was found in the urine samples collected on the third and fourth day In none of the cases was the excretion of unconjugated labelled cholic acid increased at the re examination of the patients

The ethyl acetate and butanol extracts of urine were analysed by TLC in all cases The ethyl acetate extracts contained mainly labelled glycocholic acid and small amounts of conjugates which were more hydrophobic than glycocholic acid In the butanol extracts labelled taurocholic acid and varying amounts of other labelled conjugates were detected There was no difference between the BA group and the IHC group with respect to the pattern of labelled conjugates in the butanol extracts

The percentage isotope in the ethyl acetate extract varied greatly in the individual patients (Table 3) There was no difference between the BA and IHC groups with regard to the mean percentage isotope in the ethyl acetate extracts, being 48 and 49 respectively In 4 patients with persisting jaundice (Table 4) the percentage labelled conjugates extractable with ethyl acetate was found to be higher as compared with that recorded at the first examination

After solvolysis hydrolysis and methylation of the urinary bile acids TLC analysis of all samples showed two major compounds with mobilities similar to those of methyl cholate and methyl chenodeoxycholate (Fig 2) Additional findings were small amounts of unknown compounds which were consistently found at the places of methyl esters of monohydroxycholanoic acids and in a region between methyl esters of monohydroxy and dihydroxycholanoic acids Autoradiography showed the presence of one labelled compound with the TLC mobility of methyl cholate

Half life time and pool size of cholic acid

The half life of the injected isotopic cholic acid was determined in the 5 patients with BA and in the 11 patients with IHC who excreted less than 3 per cent of the injected isotope in the faeces (Table 3) The half life of cholic acid in the former group ranged from 1 3 to 5 9 days (mean 2 6 days) the corresponding values in the latter group ranging from 0 6 to 6 2 days (mean 2 5 days) The pool size of cholic acid in the BA group and in the IHC group ranged from 13 to 46 μmol (mean 29 μmol) and 10 to 124 μmol (mean 37 μmol) respectively

DISCUSSION

Bile acid metabolism

Cholic and chenodeoxycholic acids have so far not been detected in the urine of normal human adults (29) In the normal newborn the urine was found to contain trace amounts of cholic and chenodeoxycholic acids (less than 1 μmol per 24 hours) (19) All the patients in this series excreted large amounts of cholic and chenodeoxycholic acids in the urine Great individual variation was observed The quantitative urinary excretion of cholic and chenodeoxycholic acids was virtually the same in patients with IHC who excreted less than 3% of the injected isotope and those who excreted larger amounts of the isotope in the faeces In the acute stage of cholestasis there was no difference in the urinary excretion of cholic and chenodeoxycholic acids between the

BA and IHC groups. In 2 cases of IHC associated with persisting jaundice (LB and RJ) and in 4 cases of BA (HL, KJ, CE and JS) the urinary excretion of these acids was found to be much higher in the former than in the latter at the re-examination of the patients. It is interesting to note that the ratio cholic to chenodeoxycholic acids varied greatly in both groups at the first examination of the patients. However, the ratio was found to be lower in 5 of the 6 patients at their re-examinations.

The excretion pattern of isotope after intramuscular injection of isotopic cholic acid did not differ in the cases of BA and most of the cases of IHC. However, out of the 19 patients with IHC 8 patients excreted more than 3 % of the injected isotope in the faeces at the first examination. In these cases the excretion pattern of isotope was incompatible with a diagnosis of BA.

The nature of the urinary bile acids has been previously studied in BA (18). Cholesterol-4-^{14}C was found to be mainly converted into labelled cholic and chenodeoxycholic acids which were excreted in the urine. However, the urine also contained small amounts of other labelled bile acids, which were not products of transformation of either cholic or chenodeoxycholic acids. One of these unidentified labelled bile acids was previously shown to be 3 β-hydroxy-5-cholenoic acid and was excreted in large amounts in cases of BA (14). However, the low incorporation of radioactivity into this acid from isotopic cholesterol suggested that cholesterol was not its direct precursor. It may therefore be assumed that there is a minor metabolic pathway in bile acid synthesis in BA (2, 16). All samples of urine from the patients in this series were found to contain bile acids in addition to cholic and chenodeoxycholic acids. The TLC mobility and GLC retention time indicated that one of these acids was 3 β-hydroxy-5-cholenoic acid, which was consistently found in all samples.

Conjugation of injected isotopic cholic acid did not appear to be seriously affected neither in the BA group nor in the IHC group, as labelled conjugated bile acids predominated in the urine. Even in the cases of BA and IHC with co-existent progressive liver cirrhosis the labelled unconjugated bile acid excretion was not increased at the re-examination of the patients.

Half life and pool size of cholic acid varied greatly in both the BA and IHC group. In normal infants the pool size of cholic acid has so far not been determined. For this reason the relationship between cholestasis and the latter is still unknown. The pool size of cholic acid per kg body weight was much smaller in the cases in this series than that which has been observed in normal adults (8, 13, 31). In adults with liver cirrhosis the pool size has been reported to decrease (30). There was no difference of the patients with IHC associated with congenital CMV infection and/or decreased serum concentrations of alfa$_1$-antitrypsin and the other patients with regard to the bile acid metabolism. Furthermore, the character of disturbance of bile acid excretion into bile was found to be identical in most cases of IHC with that in the cases of BA with total obstruction or absence of the bile ducts in this series. In the cases in this series the isotope excretion pattern and the urinary excretion of total bile acids was similar in character to that observed in cases of erythroblastosis complicated by cholestasis (19). The above observations suggest that cholestasis in the newborn and infants is associated with a virtually complete suppression of the bile acid excretion to the intestine irrespective whether another disease co-exists or not. On the contrary, adults with liver disease and raised serum conjugated bilirubin have not been shown to have a similar severe disturbance of bile acid excretion (1, 3, 10, 28).

Standard laboratory tests of liver function. Various standard chemical tests used for study

of the injected isotope was excreted in the faeces

In 4 patients with persisting jaundice the isotope study was repeated 10–25 weeks after the first examination (Table 4) Patients with BA were found to excrete less than 1 % in the faeces whereas those with IHC excreted 1–5 % The rates of excretion of the isotope at the re examination of the patients did not differ consistently from those observed at the first examination (Table 4)

Nature of urinary bile acids

TLC analysis showed that small amounts of labelled cholic acid often were present in the first 24 hour sample Trace amounts were detected in the second 24 hour sample from 4 of the 24 infants (KJ CR CB and ON) No unconjugated labelled cholic acid was found in the urine samples collected on the third and fourth day In none of the cases was the excretion of unconjugated labelled cholic acid increased at the re examination of the patients

The ethyl acetate and butanol extracts of urine were analysed by TLC in all cases The ethyl acetate extracts contained mainly labelled glycocholic acid and small amounts of conjugates which were more hydrophobic than glycocholic acid In the butanol extracts labelled taurocholic acid and varying amounts of other labelled conjugates were detected There was no difference between the BA group and the IHC group with respect to the pattern of labelled conjugates in the butanol extracts

The percentage isotope in the ethyl acetate extract varied greatly in the individual patients (Table 3) There was no difference between the BA and IHC groups with regard to the mean percentage isotope in the ethyl acetate extracts being 48 and 49 % respectively In 4 patients with persisting jaundice (Table 4) the percentage labelled conjugates extractable with ethyl acetate was found to be higher as compared with that recorded at the first examination

After solvolysis hydrolysis and methylation of the urinary bile acids TLC analysis of all samples showed two major compounds with mobilities similar to those of methyl cholate and methyl chenodeoxycholate (Fig 2) Additional findings were small amounts of unknown compounds which were consistently found at the places of methyl esters of monohydroxycholanoic acids and in a region between methyl esters of monohydroxy and dihydroxycholanoic acids Autoradiography showed the presence of one labelled compound with the TLC mobility of methyl cholate

Half life time and pool size of cholic acid

The half life of the injected isotopic cholic acid was determined in the 5 patients with BA and in the 11 patients with IHC who excreted less than 3 per cent of the injected isotope in the faeces (Table 3) The half life of cholic acid in the former group ranged from 1 3 to 5 9 days (mean 2 6 days) the corresponding values in the latter group ranging from 0 6 to 6 2 days (mean 2 5 days) The pool size of cholic acid in the BA group and in the IHC group ranged from 13 to 46 μmol (mean 29 μmol) and 10 to 124 μmol (mean 37 μmol) respectively

DISCUSSION

Bile acid metabolism

Cholic and chenodeoxycholic acids have so far not been detected in the urine of normal human adults (29) In the normal newborn the urine was found to contain trace amounts of cholic and chenodeoxycholic acids (less than 1 μmol per 24 hours) (19) All the patients in this series excreted large amounts of cholic and chenodeoxycholic acids in the urine Great individual variation was observed The quantitative urinary excretion of cholic and chenodeoxycholic acids was virtually the same in patients with IHC who excreted less than 3 % of the injected isotope and those who excreted larger amounts of the isotope in the faeces In the acute stage of cholestasis there was no difference in the urinary excretion of cholic and chenodeoxycholic acids between the

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ing liver disease were of little avail in differential diagnosis between BA and IHC The increase in serum LAP, GT alkaline phosphatase and transaminase activities varied in the 2 groups (Table 2) There was no difference between the cases of cholestasis associated with CMV infection decreased alfa$_1$ antitrypsin in serum, transient bacteriuria or progress to early cirrhosis and the cases not associated with these conditions with respect to the results of the standard laboratory tests of liver function

SUMMARY

This series included 24 infants 16 boys and 8 girls who were admitted to hospital with the diagnosis of obstructive jaundice Five of the infants were subsequently found to have extrahepatic biliary atresia (BA) and the other 19 infants intrahepatic cholestasis of infancy (IHC)

The infants were investigated given special attention to the quantitative urinary excretion of cholic and chenodeoxycholic acids the isotope excretion after intramuscular injection of cholic acid 24-^{14}C the nature of labelled urinary bile acids the half life and the pool size of cholic acid

At the first examination of the infants after admission the urinary excretion of cholic and chenodeoxycholic acids varied greatly between the patients However on comparing the values obtained in the two groups it was found that there was virtually no difference between the mean daily values of cholic and chenodeoxycholic acids in urine and the ratio cholic to chenodeoxycholic acid between the BA group and the IHC group After the injection of isotopic cholic acid most of the isotope was recovered in the urine in all cases In the infants with BA the faecal excretion of the isotope was low being less than 3 per cent of the injected isotope Out of the 19 infants with IHC the recovery of the injected isotope in faeces was also less than 3% in 11 infants In 8 infants with IHC the faecal isotope excretion was significantly high to exclude extrahepatic biliary atresia

The first 24 hour urine specimen contained small amounts of unconjugated labelled cholic acid in all cases whereas in no case did the patients excrete unconjugated labelled cholic acid 48 hours after the injection of the isotope No transformation of cholic acid was observed There was no difference between the BA group and IHC group with regard to the percentage labelled glycine conjugates of total excreted urinary conjugates Neither was there any difference between the two groups with regard to half life and pool size of cholic acid

There was no difference with respect to the bile acid metabolism between infants with congenital CMV infection decreased serum concentrations of alfa$_1$ antitrypsin and the other patients

ACKNOWLEDGEMENTS

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Table 1 *Clinical and laboratory findings in the 12 re examined infants*

Patient	Duration of period of jaundice (weeks)	Time of re-examination after clearing of jaundice (weeks)	Age at re-examination (weeks)	Laboratory findings at re-examination: Serum bilirubin (mg/100 ml)	Serum transaminase activity: GOT (units/ml)	Serum transaminase activity: GPT (units/ml)	BSP retention at 45 min ( )	Galactose elimination half life (min)
J R	6	1	7	0 9	55	45	n d	n d
K W	7	2	9	0 7	44	46	n d	n d
J C	7	7	14	0 5	25	15	1	n d
C R	8	8	16	0 2	46	45	27	7
M W	7	16	23	0 5	50	50	n d	n d
I J	14	3	23	0 5	148	184	12	9
M C	18	11	29	1 5	69	100	n d	10
J J	12	19	31	0 2	100	88	19	n d
S J	12	21	33	0 2	38	31	1	6
P H	6	46	52	1 2	52	40	2	8
C B	6	48	64	0 5	158	190	23	9
C N	14	58	72	0 5	131	65	n d	n d
Normal values (1 2 6) (age groups)				<1 2 (>8 wks)	18–52 (1 52 wks)	13–58 (4–24 wks) 13–38 (25–52 wks)	<5 (>3 wks)	<10 (<5 yrs)

n d = not determined

determined by the gas liquid chromatographic (GLC) method described previously (4) In order to lower the threshold of detection of bile acids the following modification was used in the controls Before quantitation of the methyl esters of bile acids by GLC these were purified by chromatography on 8.5 g columns of aluminium oxide (Woelm grade V) The residue was dissolved in benzene and transferred to the columns which were eluted with 200 ml of benzene/h vane 1 1 700 ml benzene and 200 ml ethyl acetate/methanol 1 1 Labelled bile acids were present only in the last fraction which was used for quantitation of cholic and chenodeoxycholic acids The recovery of methyl cholate 4 C after aluminium chromatography was 92% (= 7)

Alfa antitrypsin bilirubin transaminase (GOT GPT) and creatinine were determined as described previously (4) Bromsulphtalein was determined according to Varley (9) and galactose according to de Verdier & Hjelm (10)

RESULTS

Infants with Intrahepatic Cholestasis after Disappearance of Jaundice

Isotope excretion

Table 2 gives the recovering of the administered cholic acid 24-^{14}C isotope in the urine faeces and diapers i e diapers containing only ine and diapers containing both urine and faeces Only in 5 infants was it possible to isolate urine and faeces in pure form during the 4 day sampling period During that period 16–89 of the isotope was excreted In all instances most of the excreted isotope was recovered in the faeces Seven of the 12 infants excreted less than 5 and the other infants 10–26 of the administered isotope in the urine The analysis of the cumulative urinary excretion of isotope showed that in the infants who had the highest isotope level in the urine (S J and C B) most of the isotope was excreted in the first 24 hour specimen (Fig 1) In the other infants the isotope was excreted at fairly constant levels in the urine

Nature of urinary bile acids

The TLC analysis of the urinary bile acids revealed virtually no unconjugated labelled cholic acid The ethyl acetate extracts were shown by TLC to contain mainly labelled glycocholic acid The percentage of isotope present in the ethyl acetate extracts ranged from 28 to 85 The butanol extracts con

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BILE ACID EXCRETION AFTER DISAPPEARANCE OF JAUNDICE IN INTRAHEPATIC CHOLESTASIS OF INFANCY

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In intrahepatic cholestasis of infancy (neo natal hepatitis syndrome) the excretion of bile acids into bile is almost completely inter rupted Thus cholic acid 24-^{14}C given intra-muscularly is mainly excreted in the urine (4 5) Between 40 and 90 of infants with intra hepatic cholestasis appear to recover com pletely (8) but in 20 of anicteric infants bio chemical abnormalities such as raised serum transaminase and alkaline phosphatase levels were found to persist (8)

In some infants with intrahepatic cholestasis the degree of severity of the disturbance of the bile acid metabolism is essentially the same as in infants with extrahepatic biliary atresia (4) It was therefore considered to be of interest to investigate the behaviour of the bile acid metabolism after clearing of jaundice Hence we studied the isotope excretion after the ad ministration of cholic acid 24 ^{14}C and esti mated quantitatively the excretion of cholic and chenodeoxycholic acids in the urine The present paper reports the results of these studies in 12 infants They belonged to a group of 19 infants whose bile acid excretion had previously been studied during the period of jaundice (4) The results of the quantitative studies of the urinary excretion of cholic and chenodeoxycholic acids in these 12 infants were compared with those in 7 healthy infants used as controls

CASE MATERIAL

Twelve infants with intrahepatic cholestasis of infancy were re-examined 1–58 weeks after the regression of jaundice The clinical and laboratory findings at the re examination are given in Table 1 All infants were clinically healthy except infants J R and K W who showed slight liver enlargement (For further details of the case histories see ref (4)) Three infants (K W S J and J R) had congenital cytomegalic inclusion disease (CMV) and still excreted cytomegalovirus in the urine at the time of the re-examination In the other 9 infants the virus could not be isolated

Infant M C demonstrated a low serum level of alfa antitrypsin being 42 mg/100 ml i e it was in the range of Pi^{ZZ} homozygotes (normal values in adults in our laboratory are 267±84 mg/100 ml (M±2 S D)) In infants K W F H and C N the alfa antitrypsin serum concentration was slightly decreased being 168 156 and 92 mg 100 ml respectively i e it was in the intermediate range of the heterozygotes (cf 3) In the other infants the level of alfa anti trypsin was within the normal range

METHODS

Cholic acid 24 C (New England Nuclear Corp Boston Mass) 1–7 5 μCi (0 03–0 05 mg) was given intramuscularly Urine and faeces were collected daily for 4 days after the injection of the isotope All pa tients wore urinary bags and paper diapers during the sampling period (cf 4) The isotope was extracted from the urine faeces and diapers as described in a previous paper (4) The liquid scintillation technique was used for quantitative estimation of the isotope Ethyl acetate and butanol extracts of urine were analysed for free and conjugated bile acids by thin layer chromatography (TLC) and the radioactive spots were located by autoradiography (4) The urinary ex cretion of cholic and chenodeoxycholic acids was

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Patient	Duration of period of jaundice (weeks)	Time of re-examination after clearing of jaundice (weeks)	Age at re-examination (weeks)	Laboratory findings at re-examination				
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J R.	6	1	7	0 9	55	43	n d	n d
K W	7	2	9	0 7	44	46	n d	n d
I C	7	7	14	0 5	25	15	1	n d
C R	8	8	16	0 2	46	45	27	7
M W	7	16	23	0 5	50	50	n d	n d
I J	14	3	23	0 5	148	184	12	9
M C	18	11	29	1 5	69	100	n d	10
J J	12	19	31	0 2	100	88	19	n d
S J	12	21	33	0 2	38	31	1	6
P H	6	46	52	1 2	52	40	2	8
C B	6	48	64	0 5	158	190	23	9
C N	14	58	72	0 5	131	65	n d	n d
Normal values (1 2 6) (age groups)				<1 2 (>8 wks)	18–52 (1 52 wks)	13–58 (4–24 wks) 13–38 (25–52 wks)	<5 (>3 wks)	<10 (<5 yrs)

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Nature of urinary bile acids

The TLC analysis of the urinary bile acids revealed virtually no unconjugated labelled cholic acid The ethyl acetate extracts were shown by TLC to contain mainly labelled glycocholic acid The percentage of isotope present in the ethyl acetate extracts ranged from 28 to 85 The butanol extracts con

Table 2 *Bile acid excretion in the 12 re-examined infants*

Patient	Isotope excretion of administered cholic acid-24-¹⁴C: Urine (Diapers)	Faeces	Total (Diapers containing urine and faeces)	Urinary labelled metabolites extractable with ethyl acetate (glycine conjugates)	Urinary excretion of cholic (C) and chenodeoxycholic (CD) acids per 24 hours: C µmol	CD µmol	C+CD µmol	Ratio C/CD	C+CD (µmol) / Creatinine (mg)
J R	11 (5)	36	65 (18)	28	0 4	0 7	1 1	0 5	0 138
K W	10 (2)	46	89 (33)	43	1 9	0 6	2 6	3 0	0 096
J C	5 (5)	37	77 (34)	64	0 6	0 9	1 5	0 7	0 060
C R	4 (<1)	18	22 (<1)	49	0 7	0 7	1 3	1 0	0 025
M W	4 (0)	50	54 (0)	72	0 6	1 4	2 0	0 5	0 076
I J	13 (<1)	27	40 (0)	52	2 0	1 3	3 2	1 5	0 046
M C	<1 (0)	22	45 (23)	64	0 3	0 3	0 6	1 0	0 013
J J	2 (<1)	53	56 (0)	85	0 7	1 3	2 1	0 5	0 038
S J	21 (4)	51	80 (8)	56	0 8	1 1	1 9	0 7	0 022
F H	<1 (0)	14	20 (6)	74	0 7	1 7	2 4	0 4	0 020
C B	26 (15)	26	66 (14)	50	0 7	2 9	3 7	0 3	0 168
C N	<1 (0)	15	16 (<1)	64	0 5	0 8	1 3	0 6	0 027
Mean				58	0 8	1 1	2 0	0 9	0 057

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After solvolysis hydrolysis and methylation of urinary bile acids TLC analysis of all samples revealed two mnjor compounds with the mobility of methyl cholate nnd methyl chenodeoxycholate Autoradiography showed one major labelled compound which had the mobility of methyl cholnte No lnbelled methyl deoxycholnte was detected Small nmounts of labelled compounds which were more hydrophilic than methyl cholate were found in some patients

Urinary excretion of cholic and chenodeoxycholic acids

The recovery of the isotope in the urine was checked at several stages of the process of purification prior to quantitation with GLC After solvolysis and hydrolysis the final ether extract contained on average 79 of the original isotope The values of the urinary excretion of cholic and chenodeoxycholic acids (Table 2) were not corrected for losses during the process of purification

The totnl daily exretion of cholic and chenodeoxycholic ncids ranged from 0 6 to 3 7 µmol (0 01–0 17 µmol/mg creatinine) nnd the ratio cholic/chenodeoxycholic acid from 0 3 to 3 0

Normal infants

Twenty four hour specimens of urine from 7 henlthy infants aged 2–8 months were analysed In no specimen did GLC reveal the presence of either cholic or chenodeoxycholic acids after purification on columns of aluminium oxide Therefore the total dnily excretion of cholic and chenodeoxycholic acids was assumed to be less than 0 01 µmol

DISCUSSION

Previous follow up studies of infants with intrahepatic cholestasis with determinntions of serum transaminases after the regression of jaundice have shown that in 50 ∘ of infants the serum activities are elevated during the first year of life (7) In the present investigation 5 of the 12 infants showed raised serum transaminase activity at the re examinntion BSP and galnctose tolerance test could not be carried out in all infants In 4 of the 7 infants the BSP elimination was found to be con

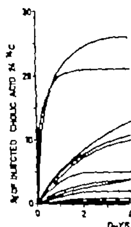


Fig 1 Cumulative isotope excretion in urine 4 days after administration of cholic acid 4 C The curves represent the isotope excretion in the individual cases From above cases C B S J I J J R, K W J C C R M W J J F H, M C and C N

siderably decreased whereas galactose tolerance tests were normal in 6 infants This indicated that the excretory function of the liver cells was impaired in some infants whereas the galactose metabolism was not affected Even in the infants who seemed clinically to be healthy the laboratory tests showed that the liver function was impaired in several cases

Studies of bile acid metabolism demonstrated that the bile acid excretion to the intestine which had essentially ceased during the period of jaundice (4) had improved In 5 of the 12 infants 10–26 of the administered cholic acid 24-^{14}C isotope was excreted in the urine This indicated that the bile acid excretion was still defective The total daily urinary excretion of cholic and chenodeoxycholic acids which ranged from 2 9 to 36 8 μmol (mean 19 2 μmol) during the period of jaundice was found to have decreased to 0 6–3 7 μmol (mean 2 0 μmol) at the re-examination of the infants There was no correlation between urinary isotope excretion and total urinary bile acid excretion The rapid urinary elimination of the isotope in 2 patients (S J and C B) was not due to an overflow of injected cholic acid since no labelled unconjugated cholic acid was excreted There was no difference between the infants with CMV infection and the other infants with regard to bile acid metabolism This applied also to the infants with subnormal serum concentrations of alfa$_1$ antitrypsin

It is interesting to note that all re-examined infants excreted bile acids in the urine whereas no bile acids were found in the urine from the controls This indicated that the bile acid excretion had not returned to normal at the time of re examination of the infants who had had intrahepatic cholestasis

SUMMARY

In twelve cases of intrahepatic cholestasis of infancy the patients were re examined 1–58 weeks after disappearance of jaundice All infants were clinically healthy but 4 infants showed raised serum transaminase

Cholic acid 24 ^{14}C was injected intramuscularly and the isotope excretion in the urine and faeces was investigated for 4 days after the injection Most of the isotope was excreted in the faeces In 5 of the 12 infants the urine contained 9–26 of the administered isotope whereas the other infants excreted less than 5 No unconjugated labelled cholic acid was excreted After solvolysis and hydrol was the major labelled compound was identified as cholic acid In 7 healthy infants used as controls no cholic and chenodeoxycholic acids were detected in the urine All the 12 patients excreted cholic and chenodeoxycholic acids in the urine at the time of the re examination the total daily urinary excretion ranging from 0 6 to 3 7 μmol These results indicated that the bile acid excretion was still impaired after regression of jaundice in the infants who had had intrahepatic cholestasis

ACKNOWLEDGEMENTS

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I J	13 (<1)	27	40 (0)	52	2 0	1 3	3 2	1 5	0 046
M C	<1 (0)	22	45 (23)	64	0 3	0 3	0 6	1 0	0 013
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The total daily exretion of cholic and cheno deoxycholic acids ranged from 0 6 to 3 7 µmol (0 01–0 17 µmol/mg creatinine) and the ratio cholic/chenodeoxycholic acid from 0 3 to 3 0

Normal infants

Twenty four hour specimens of urine from 7 healthy infants aged 2–8 months were ana lysed In no specimen did GLC reveal the pres ence of either cholic or chenodeoxycholic acids after purification on columns of alu minium oxide Therefore the total daily excre tion of cholic and chenodeoxycholic acids was assumed to be less than 0 01 µmol

DISCUSSION

Previous follow up studies of infants with intrahepatic cholestasis with determinations of serum transaminases after the regression of jaundice have shown that in 50 of infants the serum activities are elevated during the first year of life (7) In the present investiga tion 5 of the 12 infants showed raised serum transaminase activity at the re examination BSP and galactose tolerance test could not be carried out in all infants In 4 of the 7 infants the BSP elimination was found to be con

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DENTAL HEALTH OF FOUR YEAR OLD CHILDREN

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The high incidence of dental disease in childhood is well recognized all over the world (7) Although these diseases seldom endanger life they may be painful debilitating and expensive and also contribute to long term suboptimal health (7 19) Therefore these health problems should concern everyone interested in the total health of children The key to dental health lies in prevention and if prevention is to be effective an extensive proportion of the population must be reached at an early age General health service programs for children offer the best opportunities in this respect

This investigation is part of a general health control of 4-year-old children in the city of Lund and in the community of Dalby both located in the southern part of Sweden The study was designed to bridge the gap of efficient health control of children, belonging to the age groups between infancy and school age and included comprehensive medical psychological and dental investigations (22–27) This epidemiological study reports the prevalence of caries and gingivitis Based on its results a program is outlined for the future preventive dental care within the Child Health Service

MATERIAL

All children of 4 years of age living in Lund and Dalby were selected from the county population register There was a total of 1 736 4 year-old children 1 459 living in Lund 1967–68 and 27? in Dalby ... These children comprised the main study group In addition a separate group of 697 four year-old children living in Lund who had received preventive dental care for 2 years was studied as an experimental group

METHODS

The children were invited to participate by a letter to their parents The dental examination took place in the Department of Public Dentistry in Lund usually 1 3 weeks after the medical examination in Dalby on the same day as the medical examination and were performed by the same dentist (K. H)

Caries

The clinical part of the dental examination was performed with mirrors and probes (type S S White no 5) under adequate illumination (Emda Light Fantastic) with the child sitting in a dental chair Compressed air was used to dry the tooth surfaces

Because of inadequate facilities radiographic examinations were performed only on 213 children born 1963 (34 4) During the following years however two posterior bitewing radiographs were taken as a routine Satisfactory roentgenograms could be obtained in 884 of these children or 93 2 % Dental caries and restorations were recorded for each tooth surface The defs index (decayed extracted and filled surfaces) was calculated according to Gruebbel (15) The number of surfaces on teeth extracted because of caries were counted as 5 on a molar and 4 on an incisor or a cuspid Criteria of caries were those proposed by Koch 1967 (21) Teeth lost by trauma were counted as free of caries Caries diagnosed only roentgenographically was included in the defs values Based on the clinical examination an estimation of the need of treatment was made As emergency cases were considered children with need of pulpotomy or extraction of 2 or more molars in combination with 15 or more decayed surfaces

Gingivitis

The gingival status was recorded according to the gingival index system proposed by Löe & Silness 1963

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MATERIAL

All children of 4 years of age living in Lund and Dalby were selected from the county population register There was a total of 1 736 4-year-old children 1 459 living in Lund 1967–68 and 277 in Dalby 1968–69 These children comprised the main study group In addition a separate group of 697 four year-old children living in Lund who had received preventive dental care for 2 years was studied as an experimental group

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Gingivitis

The gingival status was recorded according to the gingival index system proposed by Lo & Silness 1963

(29) Gingivitis scoring was limited to second primary molars and primary incisors (12 teeth)

Malocclusion was also recorded The findings are reported in a separate paper (26)

The form used for registration of caries and gingivitis is shown in Fig 1

Interview

Immediately after the examination an interview was carried out with the mother regarding the child's eating habits oral hygiene earlier dental care and oral administration and topical application of fluoride Eating was defined as any food intake including sweets juice and biscuits The fluoride content of the drinking water was obtained from the office of the Public Dental Health Service For many of the children in Dalby this information was lacking as their water came from private wells For evaluation of the family's social standard a 3 graded socio economic grouping system widely used in Sweden was employed This pays special attention to paternal occupation group 1 representing the highest group (9)

Based on the clinical examination and the interview individual information was given to the mother about prevention of caries and gingivitis Children with caries were referred for treatment

Experimental group study

Very soon after the start of this dental health control it was evident that the prevalence of caries would b very high A caries preventive program was then introduced in the ordinary Child Health Centers of Lund implying early and regular dental supervision of the children and advice to the parents by a dentist regarding eating habits oral hygiene and fluoride administration When this program had functioned for 2 years a new year class of 4 year old children in Lund were examined i e most of these children had benefited from caries preventive measures between 2 and 4 years of age In this experimental group consisting of 697 four year old children the number of carious teeth (deft) was registered by clinical examination and bitewing radiographs were taken on those who were clinically caries free The examination was performed by the same dentist (K H)

Statistical methods

In the statistical treatment Chi square analysis in case of fourfold tables with Yates correction tests for normal distribution and regression analysis (40) were used Computations were performed at the Computer Center of Lund University (Univac 1108)

RESULTS

Main study group

In Lund 1 327 children 90 9% and in Dalby 255 children, 92 0 appeared at the dental clinic Out of the 154 children who failed to attend 63 children failed the dental examination only while the remaining 91 children did not attend any part of the health control

In Lund, the dental examination could be performed on 1 313 children out of 1 377 (98 9%) 678 boys and 635 girls, and in Dalby on 254 children out of 255 (99 6%) 129 boys and 125 girls

The findings of *caries and gingivitis* in the 1 567 children are summarized in Table 1 In Lund 26 4% were caries free while corresponding figure in Dalby was only 11 4 Emergency cases was found in 10 6 in Lund and 21 7 in Dalby The median of defs in Lund was 5 surfaces in Dalby 10 surfaces and the mean defs 7 73 and 13 07 respectively Gingivitis index in Lund was 0 35 in Dalby 0 67

On 341 children the number of decayed surfaces diagnosed by roentgenogram alone was 2 0 ± 1 16 (mean and S D)

There was no difference between boys and girls regarding caries frequency caries free children emergency cases, or gingivitis index ($p > 0\ 05$) while the difference in the same respects between Lund and Dalby was highly significant ($p < 0\ 001$)

The distribution of caries and gingivitis on different socio-economic groups is shown in Table 2

In a higher socio economic group the dental situation was better than in a lower This difference was highly significant regarding mean defs mean gingivitis index caries free children and emergency cases ($p < 0\ 001$) The best dental health was found in children whose mother had an academic education (Table 3)

In Table 4 some factors known to influence caries and gingivitis activity are listed in relation to socio economic groups and area The difference between the socio economic groups regarding neglect of tooth brushing lack of help from the parents in brushing their children's teeth frequent eating habits eating after going to bed and lack of early oral fluoride administration was highly significant ($p < 0\ 001$) being more frequent in the lower soci

Table 1. *Caries and gingivitis in 1567 four-year-old children*

	Mean def teeth	S.D.	Median def surfaces	Mean def surfaces	S.D.	Caries free children n		Emergency cases n		Gingivitis index Mean	S.D.
Lund											
Boys n = 678	4.7	4.56	5	7.91	9.78	174	25.7	70	10.3	0.36	0.36
Girls n = 635	4.6	4.62	4	7.54	9.19	168	26.5	69	10.9	0.34	0.34
Total n = 1313	4.6	4.59	5	7.73	9.50	342	26.4	139	10.6	0.35	0.35
Dalby											
Boys n = 129	6.9	4.62	10	13.08	11.76	11	8.5	25	19.4	0.69	0.49
Girls n = 125	6.8	4.90	10	13.07	12.67	18	14.4	30	24.0	0.65	0.46
Total n = 254	6.8	4.75	10	13.07	12.19	29	11.4	55	21.7	0.67	0.47

economic groups. In Dalby, neglect of tooth brushing ($p < 0.001$), frequent eating habits ($p < 0.05$) and lack of early fluoride administration ($p < 0.001$) was significantly more frequent than in Lund. In Dalby, however, the habit of eating after going to bed was less frequent than in Lund ($p < 0.05$). The fluoride concentration in the drinking water was insufficient (< 0.5 mg/l) in 90% of all children: 95.3% in Lund and 63.0% in Dalby.

To determine which factors in the children's environment and habits were most important for the caries and gingivitis activity, a multiple regression analysis was performed. The results of the interview were related to the actual findings of caries and gingivitis indices. In the regression analysis, included independable variables were symbolized by 1, 2, 3, ... 12 and excluded variables by 0. The variables are listed in Table 5.

The coefficients and t values of the regression are shown in Table 6. The terms *reduction*, *increase* and *decrease* are used to designate the difference of caries and gingivitis indices between intercept and various independent variables. The predominant effect on oral health was exerted by eating habits: children eating more than 8 times a day had on average 10.28 defs more than children eating 6 times a day or less and 5.75 defs more than children eating 7–8 times a day. The gingivitis, too, was significantly increased with more frequent consumption habits. The habit of eating after going to bed was of little importance.

The second important factor in the develop-

Table 2. *Dental findings according to socio-economic groups in 1567 four-year-old children*

Socio-economic group	def surfaces Mean	S.D.	Caries free children n		Emergency cases n		Gingivitis index Mean	S.D.
I (n = 472)	4.25	6.48	207	43.9	7	1.5	0.29	0.29
II (n = 558)	8.34	9.83	114	20.4	60	10.8	0.39	0.38
III (n = 537)	12.68	11.47	50	9.3	127	23.6	0.51	0.44
Total (n = 1567)	8.59	10.17	371	23.7	194	12.4	0.40	0.39

Table 3 *Dental findings in relation to parental education*

	Caries index		Caries free children		Emergency cases		Gingivitis index	
	Mean	S D	n		n		Mean	S D
Maternal education								
Academic (n = 317)	3 50	5 70	155	49 0	7	2 2	0 28	0 30
Non academic (n = 1279)	9 85	10 61	213	17 5	180	14 6	0 43	0 40
Paternal education								
Academic (n 515)	4 12	6 18	279	44 5	11	2.1	0 28	0 28
Non academic (n = 997)	10 82	11 04	130	13 1	171	17 2	0 46	0 41

ment of caries and gingivitis was the tooth brushing habit a significant caries reduction was achieved when the children brushed their teeth either once or several times a day A further significant decrease was demonstrated if the parents brushed their children s teeth Whether dentifrice was used or not had little influence

Early oral administration of fluoride reduced the caries activity somewhat This influence was however not statistically significant

Children in day nurseries had a reduction of caries and gingivitis activity while children in family day care generally had a small increase

Previously treated children

As shown in Table 7 42 9 of the children had previously consulted a dentist 15 4 had received conservative treatment for carious lesions and 2 9 had had teeth extracted because of caries In 18 3 or 42 3 of those

Table 4 *Oral hygiene eating habits and fluoride consumption in 1567 four year old children according to area and socio economic groups*

	Children in											
	Socio-economic group I (n = 472)		Socio-economic group II (n = 538)		Socio-economic group III (n = 537)		Lund (n = 1313)		Dalby (n = 254)		Total (n = 1567)	
	n	%	n		n		n		n		n	
Teeth brushing seldom or never	17	3 6	35	6 3	54	10 1	66	5 0	40	15 7	106	6 8
Teeth brushing without help from parents	186	39 4	230	44 8	310	57 7	613	46 7	133	52 4	46	47 6
Eating > 3 times a day	37	7 8	89	15 9	144	26 8	213	16 2	57	22 4	270	17 2
Eating every night after going to bed	27	5	58	10 4	68	12.7	138	10 5	15	5 9	153	9 8
Fluoride conc in drinking water												
0 0–0 5 mg l	457	96 8	491	88 0	463	86 2	1251	95 3	160	63 0	1411	90 0
1 0–1 mg l	3	0 6	18	3.2	25	4 7	0	0	46	18 1	46	2 9
2 1 mg l	0	0	1	0 2	0	0	1	0 1	0	0	1	0 1
Not known	12	2 6	48	8 6	49	9 1	61	4 6	48	18 9	109	7 0
Oral fluoride administration started before 2 years of age	60	12.7	4	4 3	15	2 8	91	6 9	8	3 1	99	6 3
between 2 and 4 years of age	153	32 8	180	37 3	157	29 2	433	34 7	37	14 6	482	31 4

Table 5. *Variables used in the regression analysis (see text)*

y_1 = dependent variable = caries indices = def surfaces
y_2 = dependent variable = gingivitis indices

Oral hygiene

x_1 = brushing the teeth ≥ twice a day
x_2 = brushing the teeth once a day
x = brushing the teeth seldom or never
x_3 = brushing the teeth with dentifrice
x_0 = brushing the teeth without dentifrice
x_4 = brushing the teeth with help from the parents
x_0 = brushing the teeth without help

Eating habits

x_5 = eating ≤ 6 times a day
x_6 = eating 7–8 times a day
x_0 = eating > 8 times a day
x_7 = seldom or never eating after going to bed
x_0 = every night eating after going to bed

Oral administration of fluoride started

x = before 2 years of age
x_9 = between 2 and 3 years of age
x_{10} = between 3 and 4 years of age
x_0 = no fluoride received

Day care

x_{11} = staying in family day care
x_{12} = staying at day nursery
x_0 = no day care

examined by a dentist no treatment was necessary since they were caries free. Toothache was reported for 154 children (9.8%), 58 of these (37.7%) had not consulted a dentist. From Table 7 it is also evident that children from a higher socio-economic group had more often consulted a dentist than children from a lower one ($p < 0.001$). They were also more often caries free ($p < 0.001$), they had more seldo[m] so heavy caries that extraction was indicat[ed] ($p < 0.01$) and they had less frequently suffere[d] from toothache ($p < 0.001$).

Children in Dalby irrespective of soci[o-]economic group had somewhat more ofte[n] complained of toothache than children [in] Lund (14.2% vs 9.8%, $p < 0.05$) and also faile[d] to consult a dentist when having toothache t[o] a greater extent (7.0% vs 3.1%, $p < 0.01$). Otherwise no significant difference betwe[en] the two areas was found regarding previo[us] dental care, as shown in Table 7.

Experimental group

In the group of 697 children the mean numbe[r] of deft was 3.2 ± 4.09. The number of carie[s] free children was 293 (42.0%) and the numbe[r] of emergency cases was 10 (1.4%). The dif[-]ferences of caries free children and emergency cases between the experimental group and the earlier examined children from Lund (42.0 [%] and 1.4% vs 26.4% and 10.6%) were highly significant ($p < 0.001$) as were the mean num[-]ber of carious teeth (3.2 vs 4.6).

DISCUSSION

It is evident that if WHO's definition of health as "a state of complete physical, mental and social wellbeing and not merely the absence of disease" is accepted to apply also to the

Table 6. *Coefficients and t values of the regression analysis*

	Independent variables												
	x_1	x_2	x	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}	x_{11}	x_{12}	Intercept
Y_1 *Caries* ($n = 1567$)													
Coeff. of regress.	−4.10	−3.89	−0.39	−1.19	−10.28	−5.75	−0.98	−1.15	0.44	1.80	1.09	−[illegible].16	21.37
t value	4.17	3.94	0.54	2.43	15.30	7.92	1.22	1.02	0.06	2.76	1.90	[illegible].37	
Significance	***	**		*	**	*							
Y_2 *Gingivitis* ($n = 1567$)													
Coeff. of regress.	−0.27	−0.21	−0.05	−0.07	−0.18	−0.06	−0.00	0.09	0.03	0.03	0.03	−0.07	0.52
t value	6.68	5.28	1.61	3.63	6.53	2.11	0.10	2.01	1.06	1.01	0.86	1.92	
Significance	**	**		*	**								

Table 7 *Earlier dental care reported for 1567 four year old children*

Interview	Number of children: Socio-economic group I (n 472)		Socio-economic group II (n = 558)		Socio-economic group III (n = 537)		Total (n = 1567)	
	n	%	n	%	n	%	n	%
Examined by a dentist	247	52 3	245	43 9	180	33 5	672	42 9
Received conservative dental care (restorations)	61	12 9	100	17 9	81	15 1	42	15 4
Extraction of carious teeth	6	1 3	15	2 7	25	4 7	46	2 9
No treatment necessary (caries free)	154	32 6	94	16 8	38	7 1	286	18 3
Orthodontic treatment	23	4 9	41	7 3	30	5 6	94	6 0
Toothache as cause of dental consultation	12	2 5	35	6 3	49	9 1	96	6 1
Toothache but dentist not consulted	2	0 4	18	3 2	38	7 1	58	3 7

oral cavity then very few of our pre school children are healthy The high frequency of dental disord rs is in no way unique for this country the same neglect of dental health in children is found in most countries advanced as well as developing (2 3 4 8 10 14 18 20 28 37 38 39 41 43) It is found in both sexes and in all socio-economic groups although more frequently in the lower groups (5 30 34) A detailed comparison between differ nt stud ies is made difficult by differences of meth ods criteria sampling of population and also by the inevitable subjectivity that is involved in the evaluation of caries and gingivitis Since in this study an unselected population of pre school children were examined by the same dentist some comparisons of the oral health seem justified between different subgroups of the population (e g sex area of residence socio-economic groups)

Although secondary prevention i e early treatment of carious lesions may be valuable for the children s health the ultimate goal for a dental health program is primary prevention i e prevention of the occurrence of caries There is a common agreem nt among paedo-dontists that oral hygiene di t and fluoride administration are the fundamentals in caries prevention Regarding the diet less emphasis is nowadays placed on the eating of protective and beneficial foods than on reducing the fre-quent eating of between meals and especially of sweet foods (5 6 16 44)

In a recent study from an other part of Sweden it has been shown that the nutritional requirements are well covered in this age group (36)

A nutrition study was not included in our investigation but the interview contained questions about eating habits The regression analysis showed quite clearly that frequent eating habits play the greatest role in pro ducing caries and gingivitis Neglect of tooth brushing was also an important factor It is argued (32) that removing of deposits on the teeth immediately after eating gives the best gains in caries and gingivitis reduction In our study however the frequency of tooth brush ing had only little importance in this respect Parental help in tooth brushing reduced caries and gingivitis and it is quite probable that more efficient brushing and also further im provement of oral health could be reached by more information to the parents (6 32 35)

There is an abundant literature on the benefits of fluoride in various forms of ad ministration (1 17 21 31 42) Our children had received early local and general fluoride

Table 5 *Variables used in the regression analysis (see text)*

y_1 – dependent variable – caries indices – *def surfaces*
y_2 – dependent variable – gingivitis indices
Oral hygiene
x_1 – brushing the teeth > twice a day
x_2 – brushing the teeth once a day
x_0 – brushing the teeth seldom or never
x_3 – brushing the teeth with dentifrice
x_0 – brushing the teeth without dentifrice
x_4 – brushing the teeth with help from the parents
x_0 – brushing the teeth without help
Eating habits
x_5 – eating < 6 times a day
x_6 – eating 7–8 times a day
x_0 – eating > 8 times a day
x_7 – seldom or never eating after going to bed
x_0 – every night eating after going to bed
Oral administration of fluoride started
x_8 – before 2 years of age
x_9 – between 2 and 3 years of age
x_{10} – between 3 and 4 years of age
x_0 – no fluoride received
Day care
x_{11} – staying in family day care
x_{12} – staying at day nursery
x_0 – no day care

examined by a dentist no treatment was necessary since they were caries free Toothache was reported for 154 children 9 8% 58 of these 37 7% had not consulted a dentist From Table 7 it is also evident that children from a higher socio economic group had more often consulted a dentist than children from a lower one ($p < 0\ 001$) They were also more often caries free ($p < 0\ 001$) they had more seldom so heavy caries that extraction was indicated ($p < 0\ 01$) and they had less frequently suffered from toothache ($p < 0\ 001$)

Children in Dalby irrespective of socio-economic group had somewhat more often complained of toothache than children in Lund (14 2% vs 9 8 $p < 0\ 05$) and also failed to consult a dentist when having toothache to a greater extent (7 0% vs 3 1 $p < 0\ 01$) Otherwise no significant difference between the two areas was found regarding previous dental care as shown in Table 7

Experimental group

In the group of 697 children the mean number of deft was 3 2 ± 4 09 The number of caries free children was 293 (42 0%) and the number of emergency cases was 10 (1 4) The differences of caries free children and emergency cases between the experimental group and the earlier examined children from Lund (42 0 and 1 4 vs 26 4 and 10 6%) were highly significant ($p < 0\ 001$) as were the mean number of carious teeth (3 2 vs 4 6)

DISCUSSION

It is evident that if WHO s definition of health as a state of complete physical mental and social wellbeing and not merely the absence of disease is accepted to apply also to the

Table 6 *Coefficients and t values of the regression analysis*

	Independent variables												
	x_1	x_2	x_3	x_4	x	x_6	x_7	x	x_9	x_{10}	x_{11}	x_{12}	Intercept
Y_1 Caries ($n = 1567$)													
Coeff of regress	−4 10	−3 89	−0 39	−1 19	−10 28	−5 75	−0 98	−1 15	0 44	1 80	1 09	−2 16	21 37
t value	4 17	3 94	0 54	2 43	15 30	7 92	1 22	1 02	0 06	2 76	1 50	2 37	
Significance	***	*		*	**	**				*			
Y_2 Gingivitis ($n = 1567$)													
Coeff of regress	−0 27	−0 21	−0 05	−0 07	−0 18	−0 06	−0 00	0 09	0 03	0 03	0 03	−0 07	0 82
t value	6 68	5 28	1 61	3 63	6 53	2 11	0 10	2 01	1 06	1 01	0 86	1 92	
Significance	* *	***		* *	**								

grams in other parts of the country have also led to a reduction of caries (11) Dental hygienists when available could very well accomplish major parts of these programs (33)

SUMMARY

An unselected population of 1 567 four year old children in one urban and one rural community in Southern Sweden were investigated for dental disorders as part of a general health control The mean number of defs (decayed extracted and filled surfaces) was 7 73 in Lund (urban) and 13 07 in Dalby (rural) The number of caries free children was 26 4 and 11 4 respectively and the number of emergency-cases was 10 6 and 21 7 respectively Significantly better oral health was found in higher socio economic groups A regression analysis showed that the most important factors for the prevention of caries and gingivitis were infrequent between meal eating tooth brushing with help from the parents and oral administration of fluoride at an early age in that order

An experimental group of 697 four year-old children who had got the benefit of a new caries preventing program for 2 years at the Child Health Centres showed a significant reduction of caries from 26 4 caries free children to 42 0

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administration only to a small extent and the water content of fluoride was generally insufficient (<0 5 mg/l) In the children who had received oral administration a somewhat lower frequency of caries was registered but only if it was administered from an early age

It is obvious that parents in socio economic group I had better knowledge and possibilities to protect children against caries and gingivitis more frequent tooth brushing and tooth brushing with help less between meal eating less eating after going to bed and also more frequent consultation of a dentist These differences of dental health were even more stressed if the mother s education was considered instead of the socio economic grouping which mainly reflects the father s occupation

These results are in accordance with the findings of Mansbridge (30) who states that maternal knowledge and efficiency may be more important than economic factors alone

In the early 1960 s a caries prevention program including advice on food consumption, tooth brushing and fluoride administration was introduced to schools and pre schools in the city of Lund This may explain the better oral health of children in day care centres Although this activity reached only a minority of the pre school child population it may be responsible for the better dental health in Lund compared with Dalby where no such program was performed

The rationale for the statistical analysis was not only to note factors important for the caries and gingivitis situation among the 4 year old children in these areas but mainly to let them serve as a basis for our efforts to obtain healthier children Thus it is evident from our results that preventive dental care should not start at the age of 4 years, when the vast majority of the children have already carious lesions, and when as shown in a follow up study of a sample of these children information on prevention at the age of 4 years had no influence on the caries situation 2 years later (12) Also prevention cannot be limited to small groups of the population, it must reach the majority of children and their parents

Furthermore it is also apparent that we must concentrate on factors which are amenable to action important conditions like the families socio economical and educational standards cannot be influenced within the Child Health Services

Based on the results of the present investigation an efficient program for dental health within the Child Health Service, performed at the Child Health Centre may thus be outlined as follows

1 The dentist meets the parents the first time when the child is 5–6 months old and informs them about the mechanisms behind the development of caries and gingivitis He stresses the importance of oral hygiene and in consultation with the paediatrician gives advice on food and eating habits If the fluoride content of the driking water is low he also informs about fluoride and its effect as a caries preventing agent and prescribes fluoride for oral administration

2 A second and third meeting are arranged when the child is 9–12 and 18–24 months old respectively and a clinical examination is performed if possible The information is repeated and supplemented according to the parent s questions and to the clinical findings Tooth brushing technique are demonstrated on a model or on the child Discussions are held about preventive orthodontics

3 At the age of 3 years and then once a year the child meets the dentist again either at the Child Health Centre as before or via the Public Health Service according to local conditions The information is again repeated a clinical examination is performed and carious lesions are referred for treatment or treated immediately

Gradually this program is now being introduced all over the county (13)

The reduction of caries in a following year class of 4 year olds who had tried this program from 2 to 4 years of age was considerable and highly significant Similar dental health pro

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STUDIES IN CYSTIC FIBROSIS[1]

Urinary Excretion of Hexosamine Sialic Acid and Fucose

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Cystic fibrosis of the pancreas (CF) a generalized disease with dysfunction of all or most exocrine glands is one of our most common hereditary disorders In CF there is a known disturbance of electrolyte transport with excessive excretion of sodium and chloride in the sweat (6) and saliva (7) Glycosaminoglycans and glycoproteins have been suggested to be concerned with mechanisms of ion and water transport (13 15 21 39) and the pathological behaviour of the mucus in CF has been a reason for numerous chemical studies of the mucins in different excretions

CF duodenal fluid treated with ethanol benzene gave in contrast to normal duodenal fluid a precipitate insoluble in water and not affected by trypsin (8) Further studies of the duodenal fluid showed a higher ratio of fucose to sialic acid in CF as compared with normals (10) Similar abnormal ratios were also found in saliva (3) rectal mucus (27) sweat (26) and urine (11) A higher percentage of carbohydrate was also observed in rectal mucus from CF patients (27)

One of the urinary glycoproteins the Tamm Horsfall glycoprotein is considered to originate from the urinary tract and is very easily isolated from other urinary macromolecules (35) It was reported in 1962 that this protein had abnormal aggregation properties in patients with CF (23) This finding has not been confirmed and several later investigations are unanimous that there are no qualitative differences in the Tamm Horsfall protein between normals and CF patients (5 12 30 31)

Studies of other urinary glycoprotein fractions in patients with CF have also been performed Thus Dische et al (11) isolated an ultrafiltrable nondialyzable fraction with a significantly higher fucose/sialic acid ratio than the corresponding fraction from normal urine On the other hand Talamo et al (34) found no differences either in the amount or in the distribution of different carbohydrate components in the total non ultrafiltrable urine material from normals and CF patients

Recently interest has also been focused on the glycosaminoglycan metabolism Thus Langgaard et al (18 19) showed a decreased hexosamine and uronic acid content in the skin and Matalon & Dorfman (22) reported an increase of glycosaminoglycans in cultured fibroblasts from CF patients Wiesmann & Neufeld (38) demonstrated that this increased accumulation of glycosaminoglycans in CF fibroblasts was not due to defective degradation which however was the case in fibroblasts

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Table 1 *Statistical data for the different functions drawn graphically in Figs 1–3*

	n	Mean age and range (years)	Excretion at mean age (mg/4 h)	Residual variance at mean age	Corr coeff	Slope (mg/4 h/month)	p (slope)	p (excretion)
1) *Hexosamine*							<0.05	—
Controls	38	6½ (0–20)	8.35	13.04	0.28	0.0183		
CF patients	24	7½ (0–24)	17.56	116.52	0.66	0.1142		
2) *Sialic acid*							<0.01	—
Controls	38	6½ (0–20)	6.06	7.68	0.26	0.0120		
CF patients	24	7½ (0–24)	13.83	84.23	0.73	0.1183		
3) *Fucose (secretors)*							n.s.	<0.01
Controls	27	6½ (0–14)	4.19	4.01	0.33	0.0111		
CF patients	15	7½ (0–24)	10.21	29.69	0.41	0.0347		
4) Log $\frac{\text{hexosamine}}{\text{creatinine}}$							n.s.	<0.01
Controls	38	6½ (0–20)	−1.56	0.17	0.77	−0.0079		
CF patients	24	7½ (0–24)	−1.37	0.12	0.79	−0.0052		
5) Log $\frac{\text{sialic acid}}{\text{creatinine}}$							<0.01	—
Controls	38	6½ (0–20)	−1.90	0.12	0.84	−0.0083		
CF patients	24	7½ (0–24)	−1.59	0.11	0.72	−0.0042		
6) Log $\frac{\text{fucose (secr.)}}{\text{creatinine}}$							n.s.	<0.01
Controls	27	6½ (0–14)	−2.28	0.22	0.71	−0.0073		
CF patients	15	6½ (0–24)	−1.89	0.09	0.82	−0.0067		
7) $\frac{\text{Fucose (secr.)}}{\text{sialic acid}}$							<0.05	—
Controls	27	6½ (0–14)	0.71	0.05	0.09	0.0003		
CF patients	15	7½ (0–24)	0.83	0.05	0.54	0.0070		

$$a = M_y - b\,M_x$$

M is the mean of the variable, M_x the mean age and n the number of subjects.

The correlation coefficient, r (a measure of the linear relationship) was calculated as

$$r = \frac{\Sigma xy - n M_x M_y}{\sqrt{(\Sigma x^2 - n M_x^2)(\Sigma y^2 - n M_y^2)}}$$

The variation about the regression line (measured by the residual variance) was calculated as

$$V = \frac{\Sigma(y - a - bx)^2}{n - 2}$$

The variation was described by the borders within which a new case would fall with a certain confidence. These borders, often called tolerance limits, were calculated as

$$t_{\alpha/2}\sqrt{V\left(1 + \frac{1}{n} + \frac{(x - M_x)^2}{\Sigma x^2 - n M_x^2}\right)}$$

Where $t_{\alpha/2}$ was the upper $(1 - \alpha/2)\,100$ point of Student's t-distribution with $n - 2$ degrees of freedom. The confidence level was $1 - \alpha$ and the slightly curved borders were given for a confidence of 0.95.

The comparison of two regression lines

To test whether regression lines for the control group and for the CF group were equal it had to be assumed that the true residual variances were the same. With this assumption a common residual variance was calculated as

$$W = \frac{V_N + V_{CF}}{n_N + n_{CF} - 4}$$

(N and CF indicated the control group and the CF group respectively).

The degree of non parallelism was calculated as

$$\frac{(b_N - b_{CF})^2}{\dfrac{1}{(\Sigma x^2 - n M_x^2)_N} + \dfrac{1}{(\Sigma x^2 - n M_x^2)_{CF}}}$$

Now when the hypothesis of parallelism holds, the ratio of this quantity to W is distributed as F with 1 and $n_N + n_{CF} - 4$ degrees of freedom, and was used here as a test variable.

Only when the hypothesis of parallelism was not rejected was a common slope calculated as

$$B = \frac{[\Sigma y(x - M_x)]_N + [\Sigma y(x - M_x)]_{CF}}{[\Sigma x^2 - n M_x^2]_N + [\Sigma x^2 - n M_x^2]_{CF}}$$

To test whether the lines believed to be parallel were at the same level, the following two quantities were calculated

$$\frac{((M_y - B\,M_x)_N - (M_y - B\,M_x)_{CF})}{\left(\dfrac{1}{n_N} + \dfrac{1}{n_{CF}} + \dfrac{(M_{x\,N} - M_{x\,CF})}{(\Sigma x^2 - n M_x^2)_N + (\Sigma x^2 - n M_x^2)_{CF}}\right)}$$

from patients with mucopolysaccharidosis The urinary excretion of glycosaminoglycans, which is considerably increased in patients with mucopolysaccharidosis has been reported to be within the normal range in patients with CF (4 16)

A generalized metabolic disorder such as CF might be assumed to be reflected in the chemical composition of the urine The purpose of this investigation was to try to obtain more conclusive data concerning the glycoprotein excretion in an attempt to gain additional information about the pathogenesis of CF

Since different conditions such as infections body injuries (2) and surgical operations (36) are known to influence the hexosamine excretion and since the urinary content of fucose is dependent on the secretor status and food intake of the individual (20) these factors had to be considered and standardized

Further no data about the normal excretions of hexosamine sialic acid and fucose in childhood were available and these had to be determined

MATERIAL AND METHODS

The study comprised 25 CF patients of ages 0–23 years diagnosed by clinical signs and by elevated sweat electrolytes (pilocarpine iontophoresis) as recorded in duplicate determinations A clinical evaluation of every CF patient was made with grading according to Shwachman (9) at the time of the urine collection Four patients were classified as of excellent status 6 as good 6 as mild 7 as moderate and 2 as severe Twenty one had chronic lung involvement with a productive cough and recurrent infections They were treated with daily postural drainage and all except the two oldest of them slept in mist tents and had regular or intermittent inhalation therapy They also had constant or intermittent antibiotic treatment (cloxacillin ampicillin V penicillin sulfonamides and tetracyclines) All were receiving appropriate nutritional therapy with a low fat diet pancreatic enzymes and extra vitamins including vitamins E and K The 38 controls were all healthy non hospitalized children of ages 0–20 years 13 of them being siblings from six families Special care was taken to exclude individuals with signs of infections or other illnesses at the time of urine collection

Blood group and secretor status were determined on 53 subjects as described previously (20) Since only two CF patients in this material were non secretors statistical analysis of fucose excretions was made with regard only to secretors

Urine was collected during 4 hours of starvation from children more than one year old and during 6 hours of starvation from children less than 1 year old The older children were starved for at least 10 hours and younger children for at least 6 hours before collection All infants had the same formula (Milkotal Findus Sweden) as their last meal before the starvation period

The urines were filtered and ultrafiltered at 4 C as described by Berggård (1) Visking 23 32 inch tubing was used for ultrafiltration This tube had been tested in ultrafiltration and found to retain molecules larger than about 10 000 mol wt (1) Phenylmercuric nitrate (1 50 000) was used as a preservative in all urine samples

Preparative zone electrophoresis was carried out, using the block method (17) with Pevikon C 870 as the supporting medium (24) The experiments were made in veronal sodium hydroxide buffer (pH 8 6 I 0 1) The potential gradient was 3 5 V cm and the separation time 16 hours

For *gel chromatography* Sephadex G 25 (fine) was used The fractionation procedure has been described earlier (20)

Hexosamine was determined by a modified Elson Morgan method (32) after acid hydrolysis and subsequent isolation on Dowex 50

6-deoxyhexose (fucose) was assayed by the cystein method of Dische & Shettles (9) Samples and sample blanks were included in duplicate each time

Sialic acid was determined on whole urine and ultrafiltrates using the resorcinol method described by Svennerholm (33) after hydrolysis and isolation on Dowex 2 In the eluates from zone electrophoresis sialic acid was determined by Bial s reaction using a modification of Odin (25)

Uronic acid proteins peptides and creatinine were all estimated by methods described previously (0)

STATISTICAL METHODS

Regression analysis

The linear regression equation $y = a + bx$ (y was the variable in question and x always the age) was calculated in a conventional way

$$b = \frac{\Sigma xy - nM_x M_y}{\Sigma x^2 - nM_x^2}$$

[1] This evaluation is derived from an appraisal of each of four categories 1) general activity 2) physical findings 3) nutritional status and 4) findings on chest roentgenograms Each category is given equal weight 25 points with 100 points representing a perfect score The status of the patient is considered excellent when the score is over 85 good when the score is between 71 and 85 mild between 56 and 70 moderate between 41 and 55 and severe when 40 or below

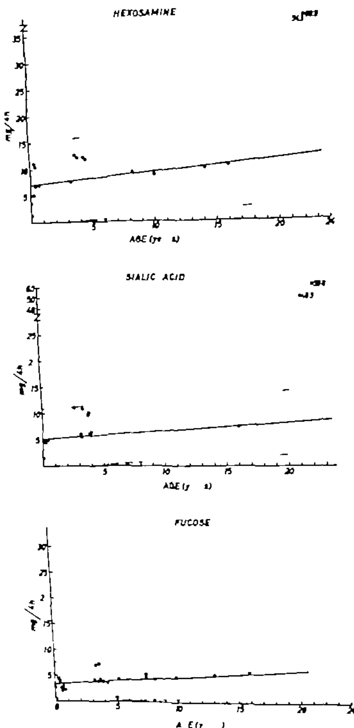


Fig. 1. Urinary excretion of hexosamine, sialic acid and fucose in CF patients and healthy controls. ○□ healthy controls (not given in Fig. 2). ●■ CF patients. (In the fucose diagrams secretors are denoted by circles and non-secretors or individuals not tested for secretor status are denoted by squares.) Mean value (—) and 95% tolerance limits ( ) for healthy children, mean value for CF patients (not given in Fig. 1).

with age matched controls. The difference was most pronounced between the two older groups. The electrophoretic patterns for these two groups are shown in Fig. 4. Each fraction contained proportionately higher amounts of fucose, sialic acid, protein and uronic acid in

and

$$\frac{(y^2 - nM_y)_N + (y^2 - nM_y^2)_{CF} - B[[\Sigma y(x - M_x)]_N + [\Sigma y(x - M_x)]_{CF}]}{n_N + n_{CF} - 3}$$

The ratio of these two quantities (distributed as F with 1 and $n_N + n_{CF} - 3$ degrees of freedom) was used as a test variable

RESULTS

The total urinary excretions of hexosamine sialic acid and fucose were determined in 24 patients and 38 controls and calculated in mg/4 h The results are plotted against age in Fig 1 and the statistical data are given in Table 1

The excretion of these three components increased with age for both controls and CF patients The excretion of fucose showed an approximately similar increase with age in controls and CF patients Thus a common slope was calculated and a comparison between the two groups showed a significantly higher excretion of fucose in all ages for CF patients ($p < 0.01$) For sialic acid and hexosamine the increase with age was more pronounced in CF patients than in controls (statistical significance $p < 0.01$ and $p < 0.05$ respectively) Thus a common slope for the excretion of these compounds was not calculated The position of the regression lines were however higher for CF patients than for controls of all ages above 2 years for sialic acid and of all ages for hexosamine

From Fig 1 it can be seen that there was a considerable individual variation in the excretions which resulted in high residual variances, and there were also low correlation coefficients for the linear regressions However by using the logarithmic functions of the ratios of these compounds to creatinine linear regression lines were obtained with high correlation coefficients and low residual variances (Table 1) Both the fucose/creatinine and the hexosamine/creatinine ratio were significantly higher in CF patients than in controls ($p < 0.01$) (Fig 2) The ratio of sialic acid/creatinine on the other hand increased more with age for CF patients than for controls This would make the calculation of a common slope misleading but the position of the regression line was higher for CF patients than for controls at all ages

The fucose/sialic acid ratio in whole urine was about 0 7 in controls of all ages In CF patients this ratio was nearly at the same level but decreased with age and since the regression lines differed significantly ($p < 0.05$) a common slope was not calculated (Fig 3)

CF patients with low urinary excretions of hexosamine sialic acid and fucose mostly came under the classification of excellent or good whereas those with high excretions were classified as moderate or severe However no statistical significance was found in this respect probably due to the smallness of the material

Ultrafiltrable and non ultrafiltrable urine fractions from five CF patients and five age matched controls were analysed for hexosamine sialic acid and fucose (Table 2) The CF patients and controls showed a similar distribution of the three components between these fractions and the two groups did not differ in fucose/sialic acid ratios either in the ultrafiltrable or in the non ultrafiltrable fraction

Urine obtained during four hours of starvation from 10 CF patients and 10 controls was pooled in four groups with 5 patients in each group Younger CF patients (3–5 years) older CF patients (14–23 years) younger controls (3–4 years) and older controls (13–20 years) The four pooled urine samples were ultrafiltered and the non ultrafiltrable material was fractionated by zone electrophoresis at pH 8 6 The eluates from the fractionations were analysed for fucose sialic acid total protein and uronic acid The amounts are expressed in mg/fraction and 4 h The fucose/sialic acid ratio of each fraction was also calculated

The total content of these components in the fractions was considerably higher in both younger and older CF patients as compared

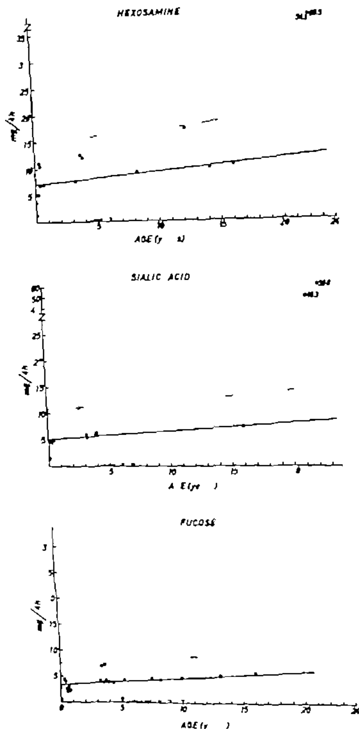


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and

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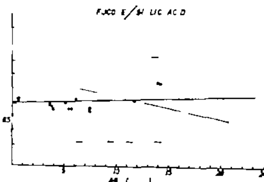


Fig 3 The ratio of urinary excretion of fucose to sialic acid in CF patients and controls according to age Symbols see Fig 1

CF patients as compared with controls and the fucose/sialic acid ratio was approximately the same in corresponding fractions from the two groups

Four hour urine samples were also collected from two starved CF patients a 4-year-old A secretor and a 3 year old O secretor Both samples were ultrafiltered and the ultrafiltrable material was fractionated on a Sephadex G 25 column The eluates were analysed for fucose The patterns obtained were qualitatively identical with those described previously for normal individuals of corresponding blood group and secretor status (20)

DISCUSSION

It has been demonstrated previously (40) that there is no correlation between secretor status and CF In this material the number of secretors (16 out of 18) was within the expected range

This report gives values of the urinary excretion of hexosamine sialic acid and fucose in normal children and in CF patients The individual excretion values show great variations and overlapping between controls and CF patients and the results must therefore be interpreted with great caution However the excretion of fucose and the ratios of fucose/

Table 2 *Percentage of hexosamine sialic acid and fucose in the ultrafiltrable fractions of urine and the fucose/sialic acid ratio in ultrafiltrable and non ultrafiltrable fractions in CF patients (n = 5) and controls (n = 5)*

	CF patients		Controls	
	Mean	Range	Mean	Range
Age (years)	7 95	3–16	7 75	3–16
Percent in ultrafiltrate				
Hexosamine	72 0	64–82	68 8	64–74
Sialic acid	81 8	79–86	77 2	70–81
Fucose	88 8	83–93	87 4	78–93
Ratio Fucose/sialic acid				
In ultrafiltrate	0 751	0 596–0 971	0 842	0 496–1 292
In non ultrafiltrable fraction	0 447	0 279–0 897	0 365	0 305–0 446

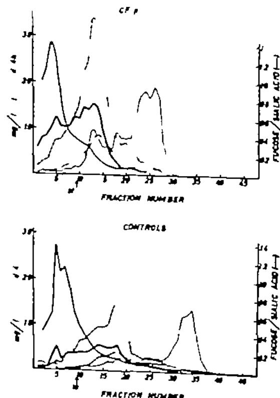
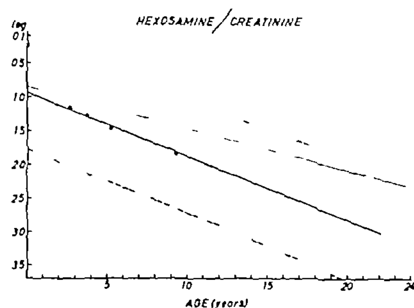
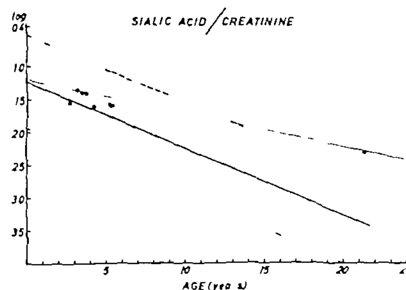


Fig 4 Fractionation by zone electrophoresis (pH 8 6) of the non ultrafiltrable material from pooled urines of 5 controls (13–20 years) and of 5 age matched CF patients (14–23 years) sialic acid — fucose (10) uronic acid (10) – – – protein — ratio fucose/sialic acid





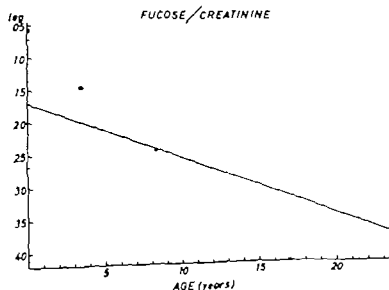


Fig 2 The logarithmic function of t ratios of the urinary excretions hexosamine sialic acid and fucose creatinine according to age *Symbo* see Fig 1

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creatinine and hexosamine/creatinine were significantly higher in CF patients than in controls of all ages ($p < 0.01$) and at least older CF patients had a higher excretion of sialic acid and a higher sialic acid/creatinine ratio Statistical analysis showed that the higher ratios were not due to a lower excretion of creatinine in CF patients

In fact the excretion of all three compounds seemed to increase more with age for CF patients than for controls Further there was a tendency to higher excretion with greater severity of the disease especially for sialic acid and hexosamine Of course these two findings are biased by the fact that they are calculated on the same material where the more severely ill CF patients were mainly found among older ages

The finding that in CF patients the excretion of sialic acid increased more with age than that of fucose was also reflected in an age dependent fucose/sialic acid ratio

The fucose/sialic acid ratio lay however mostly within the normal range in unfractionated urines Ultrafiltrates and non ultrafiltrable fractions gave fucose/sialic acid ratios within the same range both for controls and CF patients

The numerous reports of an increased fucose/sialic acid ratio in different mucous secretions do not contradict these findings since urinary fucose and sialic acid containing material is most likely derived from glycoproteins and glycolipids in serum and different tissues An increased serum level of these compounds might be secondary to different diseases such as infections where the metabolic activity is accelerated (2 36) This is also in accordance with the tendency for the excretion to be higher in more severely affected patients, in whom infections are a dominating part of the disease The possibility that the findings are explainable by primary involvement of the glycoprotein metabolism has not been ruled out however

Recently we reported (16) that there was no significant rise in the urinary excretion of glycosaminoglycans in CF children Since then Constantopoulos et al (4) have shown a slight but not significant rise in the total urinary excretion of glycosaminoglycans in 8 out of 17 CF patients (9–25 years old) The urinary excretion of glycosaminoglycans from CF patients in our material was in total agreement with their findings (37)

The study has not revealed any qualitative differences in the hexosamine sialic acid fucose or uronic acid-containing material in urine between CF patients and controls However it cannot be excluded that some minor components may be qualitatively different in CF patients and possibly also may be detected by more extensive subfractionation of the material

SUMMARY

Normal values for the total urinary excretion of hexosamine sialic acid and fucose and their ratios to creatinine in childhood were determined The excretion of these substances in 25 CF children was slightly higher at all ages than in controls The increase was more pronounced in higher ages and in more severely affected patients

No difference in the ratio of fucose to sialic acid was found between controls and CF patients either in the unfractionated urine or in the ultrafiltrable or non ultrafiltrable fractions of urine

Zone electrophoretic fractionation of the non ultrafiltrable material disclosed a proportionately higher excretion of both carbohydrate and protein containing material in CF patients as compared with controls

Two CF patients exhibited normal gel chromatographic distributions of fucose-containing low molecular weight carbohydrate material

ACKNOWLEDGEMENT

The authors are indebted to Mrs G Pettersson for valuable technical assistance

Acta Pædiat Scand 62 289–296 1972

ACCIDENTS AND SURGICAL EMERGENCIES IN A POPULATION OF MENTALLY RETARDED CHILDREN

SHEILA MARGARET BERGGREEN

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Many scientific works have been written about the incidence of accidents in children This is evidenced by the comprehensive list of references following the thesis by Kolle Jørgensen (5) on accidents in a population of normal Danish children In this thesis the circumstances involved in the occurrence of the accidents are analysed Other articles are concerned with the hazards to which children are exposed at home and at play e g the leaflet published in 1968 by the American Committee on Accident Prevention (2) Understandably this topic recurs regularly in the popular press and other mass media

As far as I am aware no investigations have hitherto been undertaken on populations of mentally retarded children

This pilot study was undertaken to review and investigate retrospectively the casualties and surgical emergencies which occurred during the 3 year period 1968 1969 and 1970 in the Children's Hospital in Vangede It was hoped that the results of the investigations would provide information about the causes of these accidents and their prevention and illustrate the phenomenon of accident proneness if such a condition exists

MATERIAL

The Children's Hospital in Vangede provides long term resident accommodation for 276 mentally retarded patients accommodated in homes for from 12 to 15 children In addition there is a sickbay with accommodation for 15 patients and 20 spastic patients living in their own homes receive day-care

This article was read in an abbreviated form at a postgraduate course held by The Danish Society for Mental Deficiency Research in Århus in March 1972

The population from which this material originates is thus slightly over 300 patients This population is not altogether static In the wards minor fluctuations occur while in the sickbay short term cases are mixed with long stay patients awaiting more permanent placement elsewhere The ratio of boys girls in the basic population was approximately 3 : 2 In 1969 the middle year of the period in question the average age was 9.5 years

Further information about the Children's Hospital in Vangede may be obtained in a descriptive leaflet (1)

The hospital has a casualty department with facilities for treatment of minor injuries and for radiography Medical help is always available Serious casualties (e g where anaesthesia is required in the treatment etc) can be transferred to the local general hospital A number of casualties never reach the casualty department as suitable treatment can be provided in the wards The figures quoted here are thus minimum figures

METHOD

The information obtained originated from three main sources (i) the casualty records for the three year period (ii) the X ray records, and (iii) in cases where the children had been treated or admitted elsewhere from letters of discharge and from the case histories A questionnaire was sent to each of the wards to enquire about such cases and this was followed up by a personal interview with as many members of the staff as possible It is possible that a number of cases in this category evaded our notice and the figures obtained are thus minimum figures again

Limits of the investigation

This investigation takes into account surgical emergencies and casualties only Planned surgical inter

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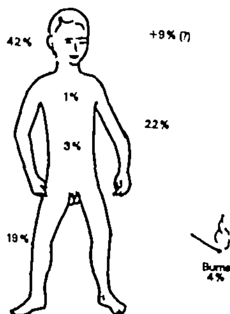


Fig 1 Percentage distribution of injuries

ventions and emergencies requiring consultant specialist treatment (dental ophthalmological and ear nose and throat) were excluded Similarly medical emergencies such as ingestion of poison infections etc were also excluded

Procedure

A punch-card system was employed and a card was made for *each casualty* with a brief summary of the following data date time name age ward nature and localization of the injury and the treatment employed The cards were then punched according to codes to indicate sex age diagnosis intelligence quotient relevant complicating somatic disease handicaps of the special senses (sight hearing and possibly anaesthesia) mobility and whether iatrogenesis carelessness or prophylactic measures had played any part in the mechanism of the accident The records were not invariably complete and it did not prove possible to obtain comprehensive records of the times at which the accidents occurred In a number of cases even the site of the injury was unrecorded These latter cases were therefore coded under a special heading site unknown

RESULTS

The total number of injuries in the 3 year period was 408 If dental injuries had been included, this number would have been almost doubled (9) This figure is approximately three times the figure found by Kølle Jørgensen (5) in his medico social investigation of accidents in normal children in Odense The Odense material however included a number of *medical* emergencies (e g cases of poisoning) which were excluded in the present review The incidence of casualties in mentally retarded children is thus probably considerably more than three times the incidence in a normal child population despite the fact that these retarded children were living in a sheltered environment

In the Children s Hospital which consists of 21 wards or houses the maximum number of accidents (12 5 * of the total) occurred in a ward for teenage (10–15 years) children suffering from severe epilepsy In this ward 6 particular children were found to be the most "accident prone and were together responsible for 39 out of the 50 accidents in the period Epileptic seizures undoubtedly played a major role in the causation of these accidents and it is interesting to note that several of these children repeatedly sustained the same type of lesion e g one boy sustained repeated injuries to the soft tissues around the mouth (and the teeth) while another repeatedly sustained lacerations of the chin This phenomenon is probably connected with the pattern of the epileptic seizure On account of the repeated injuries at more or less the same site, surgical repair becomes increasingly difficult on account of scar tissue The cosmetic results become progressively poorer and may where injuries near the mouth are concerned be further impeded by gingival hypertrophy caused by the anticonvulsants administered which may make the tissues in this region more friable and liable to injury (8)

Two other wards were each responsible for 8 of the total number of injuries One of these wards is for profoundly and severely retarded children several of whom are mongols and all of whom are relatively active and here the injuries varied greatly in nature *The other* ward is for teenage children several of whom are epileptics and here again epilepsy appears to be the major predisposing factor Six other wards were each responsible for 5 or 6 of the casualties and the remaining 37 5 were more or less uniformly distributed among the

12 remaining wards. Relatively few injuries occurred among autistic children.

Localization and Type of Casualty

Injuries of the head and face (lacerations of the scalp and face, fractures and concussion) constituted 42 of all the injuries. This figure is possibly nearer 50% as the records concerning the localization of 9 of the casualties were incomplete and these were therefore recorded under the heading site unknown. It is suspected, however, that the majority of these were lacerations of the face or scalp. The vast majority of the casualties involving the head and face were minor lacerations for which one or two sutures sufficed. The sexes were found to be equally represented. The peak ages for head injuries were 9–11 years. In normal children, Kølle Jorgensen (5) found peaks for similar injuries at much earlier ages. This difference is probably explained by the slower motor development of the retarded children. Epilepsy and cerebral paresis, singly or combined, were present in about 80% of the boys and 70 of the girls who sustained head injuries. A striking preponderance of Down's syndrome among the girls was observed. This was not explained by overrepresentation of mongol girls in the original population but appeared to be due to a little group of particularly active mongol girls. Low intelligence quotients preponderated among the children sustaining head injuries. The majority of patients with head injuries could walk alone or with some support but they were frequently unsteady in their gait. A group of 5 hydrocephalic boys proved to be an exception to this. They sustained frequent head injuries, occasionally with symptoms of concussion, although they were all wheel-chair patients. The mechanism involved appeared to be loss of balance on account of their large heavy heads and poor muscle tone. On account of the hydrocephalus their heads were possibly simultaneously more vulnerable so that concussion resulted from injuries which would not have caused this in normal heads.

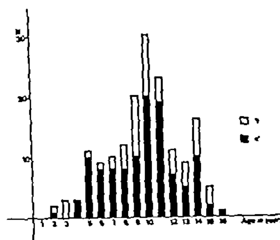


Fig. 2. Injuries to the head and face. Age distribution showing peak incidence several years later than in a normal child population.

Impairment of sight and hearing were possibly contributory factors in a few cases. The most serious head injury was fracture of the skull in an active 11 year-old girl with Down's syndrome following a fall out-of-doors. Nearly half of the patients sustaining head injuries were equipped with protective helmets but unfortunately our records do not always state whether these were worn at the time of the accident. On some occasions the injury occurred while the child was being bathed or dressed in the morning and a *combination of factors* such as a slippery hard bathroom floor and a convulsion in a child whose blood level of anticonvulsants was at its lowest ebb and whose helmet had not yet been put on appears to have occurred. Aggression on the part of a fellow patient probably played a part in some cases but the records are unfortunately incomplete.

Injuries to the upper limbs. These constituted 22 of the total and were classified into three groups: soft tissue injuries, fractures and injuries of the fingertips. The age and sex distribution of these accidents correspond to those in the original population. The vast majority of children sustaining these injuries were profoundly or severely retarded and defective sight and hearing appear to have played

minor roles in some cases Here again epileptics and spastics who could walk with or without support appeared to sustain these injuries more frequently than patients in other groups Injuries to the fingertips comprised 27% of the upper limb injuries and were usually caused by crushing of the fingertips in the hinges of doors or windows Occasionally almost *complete indifference to pain* was observed in these patients and corresponded in distribution to cerebral paresis Some fractures of the fingers and metacarpals were due to children getting their hands caught in the wheels of invalid-chairs or prams etc and were thus an upper limb equivalent of the bicycle wheel lesions of the feet of normal children described by several authors and quoted by Kølle Jørgensen (5) A slight preponderance of fractures was observed in girls

Injuries to the lower limbs These comprised 19% of the total injuries and were similarly classified into three groups soft tissue injuries fractures and injuries to the toes Injuries to the toes were however rare (only three) but in two of these relative indifference to pain appeared to be present Here again epilepsy cerebral paresis and low intelligence quotients appeared to be predisposing factors Several *spontaneous fractures* occurred in helpless bed ridden patients and X ray examination revealed extreme halisteresis in all of these In two cases fractures occurred as complications of orthopaedic interventions In both of these the bones were more slender than normal for the age In girls the majority of lower limb accidents occurred in patients who could walk with or without support whereas a number of boys in wheel chairs sustained injuries to the lower limbs A boy who crawled on his knees developed recurrent prepatellar bursitis In boys the fractures were concentrated around the knees and two cases of hydrarthron of the knee joint were observed The numbers are so limited that no conclusions appear permissible from this observation

It has been suggested that the calcium metabolism in epileptic children receiving anticonvulsants is altered and that this results in hypocalcaemia or occasional osteomalacia (4) The present investigation could neither confirm nor refute this postulate

Burns were classified separately irrespective of the site and comprised just over 4% of the total material Several cases of quite extensive *sunburn* with blistering occurred in helpless patients who were exposed to early summer sunshine Three cases of *chemical burns* due to undiluted disinfectant were recorded Two of these were due to accidental splashes and the third occurred in a 15 year old autistic girl who collected discarded Rodalon® containers in the pocket of her jeans and sustained a superficial chemical burn on the thigh Two children a boy and a girl were accidentally *scalded* on the trunk by boiling water in a nursery school A boy with the Cri du chat syndrome sustained *burns* of the tongue and lips by licking the radiator in his room A teenage girl (I Q about 70) with anaesthesia of one lower limb resulting from an extensive lumbar myelomeningocele sustained a large second degree burn by sitting with her knee against a washing machine in her home The remaining burns were all much less extensive There was a preponderance of burns in girls possibly because they frequented the kitchens more

Thoracic and Abdominal Conditions

Thoracic conditions

Only those requiring surgical treatment are included here Three such cases occurred A girl with the Cornelia de Lange syndrome developed acute dysphagia and investigation in a general hospital revealed a congenital short oesophagus A profoundly retarded girl with the Happy puppet syndrome developed massive empyema of one entire lung with displacement of the mediastinum and was successfully treated by surgical decortication of the lung in the local general hospital An epileptic boy had a convulsion during a meal and inhaled a piece of egg which was removed by bronchoscopy in a general hospital

Abdominal conditions

As these varied greatly in nature they will be described in greater detail

Torsion of an ectopic testis occurred in an epileptic boy aged 15 with athetotic tetraplegia and extreme extensor thrust He was secured in his wheel chair by groin straps and it is thought that the repeated traumata caused by these straps on the ectopic pubertal testis resulted in its torsion

A massive haematoma of the abdominal wall and right thigh in a profoundly retarded boy of 12 years was thought to be due to a similar mechanism In this case the trauma exerted by the straps was on enlarged inguinal glands following insect bites on the leg

Acute appendicitis occurred in a 15 year-old boy Operation revealed a gangrenous appendix

Acute retention of urine occurred on various occasions in 4 boys Two of these had phimosis one had pubertas praecox and priapism and the fourth patient was moribund

Swallowed foreign bodies This was a frequent occurrence and many cases probably escaped our notice entirely Only cases with complications will be recorded here A profoundly retarded teenage boy with Down's syndrome habitually ate everything within his reach Over a period of months he lost considerable weight and had frequent bloody motions A pelvic mass was felt and thought to be neoplastic Laparotomy revealed an abscess containing a variety of foreign bodies which had ulcerated through the posterior wall of the pelvic colon An 8 year-old profoundly retarded Turner mosaic girl similarly ate everything She had frequent attacks of threatened intestinal obstruction which could usually be relieved by enemata followed by the passage of diverse foreign bodies Three other children swallowed dental appliances These were followed radiographically and were passed uneventfully *per vias naturales* Thus with one exception ingested foreign bodies in mentally retarded children were managed conservatively but vigilance is essential (11)

Haematemesis was a surprisingly frequent occurrence in this clientele No less than 9 cases occurred during the period in question In 5 of these haematemesis was a terminal event One boy had oesophageal varices and 4 other cases were probably due to congestive cardiac failure Four patients who are still alive have occasional blood stained vomits for which no adequate explanation has been found Hiatus hernia with peptic ulceration at the lower end of the oesophagus is a possible explanation but has not been proved Similar observations have been made by Dahl among mentally retarded children in Ebberødgård (3)

Perforation of the small intestine This was the only traffic accident in the material A 14-year-old boy was struck on the left lower abdomen by the handlebar of his bicycle The following morning signs of shock and peritoneal involvement were present and X ray revealed pneumoperitoneum Perforation of the small intestine was found and closed The mechanism in this case is comparable to that described in an article on steeringwheel injuries by Larsen & Vestergaard (7) and the finding of pneumoperitoneum after a latent interval was of diagnostic significance

Self inflicted injuries One of the original objects of the investigation had been to investigate these It proved difficult however to delimit them Cases in which the injuries required therapy were recorded but it subsequently beame obvious that a number of cases existed where slight but repeated injuries were inflicted so that chronic reactions occurred Cases such as these did not necessarily reach the casualty department unless the lesions became infected The amount of information available varied with the attitude of the ward leader and the type of patient in the ward The types of self mutilation encountered varied from simple "bad habits such as finger sucking and nail biting to much more grotesque forms such as head banging pulling out hair biting kicking and scratching The information gained seems to indicate that nail biting and finger sucking were observed in children

with relatively high intelligence quotients while the more grotesque and violent forms appear to occur in profoundly retarded patients Occasionally the vicious circle appears to have been initiated by a transient local irritating lesion or skin condition and the habit has persisted long after the original cause has disappeared As in blindisms or the habits of some blind persons to hit rub or poke the blind eye it may be possible that partial or complete anaesthesia or paraesthesia of the region involved is present and that the apparently violent self mutilation produces a pleasurable sensation Self inflicted injuries became infected more frequently than accidental injuries Sørensen (10) made similar findings in her analysis

DISCUSSION

The casualties presented here correspond by and large to accidents in the home The children involved live under sheltered conditions with a maximum of skilled supervision and are protected from many of the hazards to which normal children are exposed e g tools traffic and the competitive rough and tumble of playgrounds Parents frequently express considerable indignation that accidents can happen while their children are under our care It is hoped that this investigation will throw some light on the problem

The incidence of accidents in this material is three times that in the normal Danish child population and the same as that found by Zachau Christiansen (12) in Greenland viz a population living under primitive conditions with many natural hazards

Firstly the positive findings will be summarized Only one traffic accident occurred during the 3 year period Despite unrestricted but supervised play in paddling pools during the summer months no drowning accidents occurred Very few accidents resulted from play with tools and there were no fatal accidents No electric burns occurred probably on account of the vigilance of the staff and the fact that the wall contacts were protected by special plugs when not in use Sepsis was rare probably because of easy access to primary suture

The majority of accidents described here are of types which normally occur in toddlers Because of the delayed motor and mental development in our clientele these accidents occur here in a considerably older age group and the consequences are more serious because the children are heavier and clumsier The incidence of accidents could of course be reduced by confining the children to bed or restraining their activities but this would result in further retardation of their development and accidents would be replaced by the consequences of prolonged immobility e g bed sores halisteresis urinary infections pulmon infections and mental apathy

Epilepsy is shown to be a major predisposing factor particularly to head injuries Medical treatment of epilepsy is therefore of paramount importance

Where severe epileptics and spastics are concerned the environment must be adapted to the needs of the patients It is quite ridiculous to talk of normalizing their surroundings Helmets and even mouth protectors although unsightly must be obtained and used Epileptics who have seizures in the mornings and which cannot be controlled by alteration in the times of administration of anticonvulsants should be allowed to stay in bed for breakfast so that seizures if any occur in bed Resilient floor coverings such as felt carpeting or linoleum with felt or foam backing are advisable and non slip materials must be incorporated in bathroom floors which are normally slippery when wet Large mattresses for playing on and element furniture consisting of foam rubber or plastic blocks and chairs and cushions filled with plastic pellets or similar material are good investments The corners and hard edges of existing furniture should be rounded smoothed and possibly padded A recent American report on residential services for the mentally retarded (6) observes that hygienic demands must sometimes be sacrificed for the welfare of the patients

Once ideal surroundings have been obtained maximum activity can be encouraged and overprotection which may be just as dangerous as carelessness avoided Accidents will continue to occur and these must be accepted as a painful but necessary part of the development of the handicapped child as is the case in the normal child

A considerable number of the fingertip accidents occurred by crushing in the hinges of doors and windows and the question is raised whether folding doors should be employed in future institutions An extra door handle high up on the door out of reach of the children is a cheap and effective solution on existing doors

Groin straps should be avoided in boys with cryptorchism or in children of either sex if there is any suggestion of inguinal adenitis

The investigation revealed that our records are incomplete It is of value to record the time of the day at which the accident occurred as this knowledge may be employed therapeutically It is also of interest to know whether the prophylactic measures prescribed were actually in use when the accidents occurred to assess their value The circumstances of the accident should be recorded as they may indicate aggression on the part of an other patient or stress among the staff It is also relevant to know where the accident occurred both from the point of view of therapy (e g booster doses of tetanus globulin) and to disclose other sources of danger

SUMMARY

The incidence of casualties in an institution for mentally retarded children is shown to be about three times as great as in the normal Danish child population The circumstances concerned are analysed by means of a retrospective investigation to elucidate the mechanisms involved and the contributory factors

The investigation reveals that there is a definite relationship between the degree of mental handicap and the underlying disease (e g epilepsy and/or cerebral paresis mongolism etc) and the incidence of casualties Other types of incapacity such as motor handicap anaesthesia defective sight or hearing and other physical disease (e g hydrocephalus) may predispose to accidents Some children are undoubtedly more accident prone than others and the cause may be found in their disease (most frequently epilepsy) while in others purposeless motor activity and the inability to learn from experience combined with severe or profound mental retardation appear to be responsible Phases of accident proneness may be associated with the acquiring of new skills

Some prophylactic measures are suggested which should be considered when new institutions are planned

A broad spectrum of somatic developmental handicap environmental emotional and mental factors interplay in the production of accidents in mentally retarded patients Attempts should be made to analyse the mechanism of every accident so that appropriate prophylactic measures can be undertaken

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Table 2 *Incidence of some signs of possible nutritional significance*

Group	Cheilosis		Angular stomatitis		Bleeding gums		Hypertrophic interdental papillae		Skin xerosis	
	n		*n*		*n*		*n*		*n*	
Lysine	8	4	4	2	16	8	22	11	50	25
No Lysine	3	3	1	1	6	6	13	14	22	24
Control	2	2	2	2	13	11	15	13	28	25

Table 3 *Weight height and mid arm circumference for age*

Group	Weight[a]		Height[a]		Mid arm circumference[b]	
	n		*n*		*n*	
Lysine	142	70	162	80	97	47
No Lysine	70	78	77	86	45	50
Control	85	74	106	92	53	46

Under the 10th percentile (20)
[b] Under 90 of value given by O Brien et al (22)

km south of Teheran were subjects of our study The general conditions health standards and diet in villages of this type and in this region have been described elsewh re (12)

Th children were divided into three groups Lysine (Sharifabad Ibrahimabad and Abdollabad) No Lysine (Kolin) and Control (Karimabad) The numb r of subjects in each group and their distribution by age and s x is given in Table 1

METHODS

The children in the three groups were examined clinically The frequency of signs of possible nutritional significance is given in Table 2

Table 4 *Composition and nutritive value of meals*

Meal	Amount served (g)	Times served	Protein g	Protein A[a]	Kcal 10³	Kcal A	Calcium g	Calcium A	Iron mg	Iron A	Thiamin mg	Thiamin A
Halva ardeh Butter Bread	100 25 350	30	43	61	1 6	63	0 7	58	32	100	0 8	60
Ashe jow Bread	300 350	28	36	52	1 0	41	0 7	58	31	100	0 9	70
Borsh Bread	250 350	8	35	50	1 0	41	0 7	58	30	100	0 9	70
Tass kebab Bread	250 300	24	35	50	1 1	43	0 7	58	30	100	0 9	70
Reshteh polow Bread	200 300	15	34	50	1 2	50	0 6	50	29	100	0 9	70
Khoreshte Sabzi Rice Bread	 100 200 300	26	36	52	1 2	50	0 5	45	4	100	0 8	60
Ashe alou Bread	250 350	30	34	50	1 0	41	0 6	50	30	100	0 9	70
Khoresht Kadow Bread	 150 350	14	33	47	1 1	43	0 6	50	24	100	0 8	60

[a] Percentage of daily allowance provided by the meal according to N R C

Weight height and mid arm circumference were obtained by two of the authors (H Sh and G D). Weight males wearing only underpants and females shirt and pantaloons were measured to the nearest 100 g. The same beam balance was used throughout the trial and was checked repeatedly each day at 0 and 70 kg with the same certified weights. Height all the subjects were measured to the nearest 0 5 cm. The height of the beam was repeatedly checked with a metal rod at 150 cm. Mid arm circumference was measured with a flat metal tape as suggested by Jelliffe (18).

The percentage of children from each group under the 10th percentile of weight and height for age (20) and under 90% of the standard mid arm circumference for age (22) are given in Table 3.

Blood was drawn and haemoglobin (17) and serum albumin (3) determined.

FEEDING

A feeding programme was designed and implemented to provide about 50% of the daily protein and calorie recommendations and a substantial proportion of the mineral and vitamin allowances (7) and given 6 days per week over a period of 205 215 days to the children from the Lysine and No Lysine groups. The Control group received no extra food.

Menus were designed in such a way to include typical Iranian culinary preparations from which animal protein and legumes were excluded and to some of which sesame was added. A portion of bread was offered in the morning at breakfast (about 150 g). The culinary preparations were served at lunchtime together with another portion of bread (about 200 g). The composition and nutritive value of the meals offered is shown in Table 4.

Bread was baked locally from Iranian wheat flour of 95% extraction which was enriched under our direct supervision with a pre mix that contained a vitamin and mineral mixture (No Lysine group) or with the same mixture plus L Lysine hydrochloride to provide a level of about 2 5 g of L Lysine base per kg of flour (Lysine group). Chemical and biological tests with rats showed the amino acid to be available after the baking of the bread. The addition of lysine considerably increased the utilization of nitrogen (as NPUop) and the weight gains to rats that received bread enriched with the amino acid (10). Table 5 indicates the chemical score of the protein from the school meals using modified FAO 1957 Reference Protein (5) and egg (6) calculated from amino acid analyses of the meals. Amino acid analyses of the meals showed the protein to be limited by its lysine content.

No attempt was made to control the food intake of the children at their homes.

ASSESSMENT

At the end of the feeding trial the children from the three groups were subjected to the same anthropometric measurements and biochemical determinations as they had been at the beginning.

Riboflavin		Niacin		Vitamin A			Ascorbic acid	
mg	A	mg	A	U 10³		A	mg	A
1 5	82	14	83	1 0		23	35	47
1 6	88	15	88	3 5		77	44	59
1 6	86	16	93	4 9		100	40	53
1 6	89	16	93	4 2		93	40	53
1 5	82	13		2 8		61	37	49
1 4	79	13	78	2 7		59	45	60
1 8	99	—	—	—		—	—	—
1 3	97	—	—	—		—	—	—

RESULTS

The changes in weight height and mid arm circumference are given in Table 6.

The children who did not receive lysine showed larger increments in height and weight than those whose diets were supplemented with this amino acid. However the difference observed was significant only for height. Significantly higher ($t = 3\ 05$ $p = 0\ 01$) increments in mid arm circumference were shown by children who received lysine supplementation.

See Appendix I

Tryptophan 60 mg/g N

Amino acid analyses of bread and meals kindly performed by Prof P L Pellet (American University of Beirut) Central Laboratory D.S.M. Heerlen (Netherlands) Central Institute for Nutrition and Food Research T.N.O. Zeist (Netherlands) and Astra Nutrition Molndal (Sweden)

Table 5 *Protein score of school meals and effect of lysine addition*

	Protein score	
	Egg	FAO reference protein
Halva ardeh + bread	36 (LYS)[b]	58 (LYS)
Halva ardeh + (bread + Lys)	51 (LYS)	82 (LYS)
Ashe jow + bread	46 (LYS)	73 (LYS)
Ashe jow + (bread + Lys)	6[illegible] (THR)	84 (S)
Borsh + bread	42 (LYS)	67 (LYS)
Borsh + (bread + Lys)	56 (THR)	79 (S)
Tass kebab + bread	37 (LYS)	60 (LYS)
Tass kebab + (bread + Lys)	56 (THR)	78 (S)
Reshteh polow + bread	41 (LYS)	67 (LYS)
Reshteh polow + (bread + Lys)	56 (LYS)	85 (S)
Khoreshte sabzi + rice + bread	41 (LYS)	70 (LYS)
Khoreshte sabzi + rice + (bread + Lys)	57 (LYS)	90 (S)
Ashe alou + bread	41 (LYS)	66 (LYS)
Ashe alou + (bread + Lys)	56 (THR)	78 (S)
Khoreshte kadow + bread	40 (LYS)	65 (LYS)
Khoreshte kadow + (bread + Lys)	57 (THR)	80 (S)

[a] Modified TRY − 60 mg/g N
[b] Limiting amino acids denoted in parentheses

The weight gains and increments in arm circumference were significantly greater in the children who received school meals (Lysine or No Lysine) than in those who did not (Control) Also the gain in height was larger but

Table 6 *Changes*[a] *in weight height and mid arm circumference*

Group	n	Weight (kg)	Height (cm)	Mid arm circumference (cm)
Lysine (L)	203	0 97 ± 0 060	2 8 ± 0 071	0 49 ± 0 048
No Lysine (NL)	90	1 21 ± 0 103	3 1 ± 0 116	0 23 ± 0 071
Control (C)	115	0 12 ± 0 062	2 7 ± 0 073	0 03 ± 0 056
		t p	t p	t p
L vs NL		1 96 NS	2 31 0 025	3 05 0 01
L vs C		10 0 0 001	0 95 NS	6 40 0 001
NL vs C		15 0 0 001	3 05 0 01	2 22 0 025

[a] Mean ± S E M

Table 7 *Changes*[a] *in serum albumin and hemoglobin*

Group		Serum albumin g/100 ml		Hemoglobin, g/100 ml	
		Before	After	Before	After
Lysine	n	169	169	167	167
	m	3 99	3 68	14 04	13 97
	S E M	0 035	0 041	0 067	0 083
Before vs after	t	5 64		0 66	
	p	0 001		NS	
No Lysine	n	81	81	75	75
	m	4 11	4 77	14 79	14 61
	S E M	0 054	0 077	0 111	0 1[illegible]4
Before vs after	t	1 62		1 92	
	p	N S		NS	
Control	n	69	69	67	67
	m	3 83	3 96	14 20	14 41
	S E M	0 504	0 411	1 07	1 18
Before vs after	t	1 64		1 46	
	p	NS		NS	

Mean ± S E M

the difference reached significant levels only with the group that received no lysine The changes in serum albumin and hemoglobin are given in Table 7

A small but statistically significant decrease in serum albumin level was evidenced by the children who received the amino acid addition (L) No significant differences were observed in those that did not receive lysine supplementation (NL) or in the controls that received no school meal (C)

DISCUSSION

There is no doubt that the addition of Lysine to cereals improves the protein value of these foods However the intake of lysine from other food items (pulses legumes animal products) in human diets can render the sulfur amino acids—rather than lysine—the first limiting factor or considerably reduce the distance between the level of limitation due to lysine and the next limiting factor (21) If sulfur amino acids are the first limiting amino acids of the value of protein no benefit will be obtained from the addition of lysine Furthermore if the level of limitation due to other amino acids is very close to that of

lysine little can be expected from the sole addition of this amino acid The diets given were purposely designed to be limited in lysine This was achieved by excluding animal protein and pulses in the formulation of the culinary preparations Furthermore in some meals the distance between lysine and sulfur amino acids was stretched by adding sesame an item that contains a high proportion of the latter These measures aimed at offering a diet to which the addition of lysine would mean an improvement in protein quality It can be seen from Table 5 that an average sixteen units of chemical score (egg or FAO 1957 modified) could be expected by the addition of lysine Results from surveys performed in different regions of Iran (including the area where this study was conducted) show the diets to be limited by lysine or sulfur amino acids depending on whether the modified FAO provisional pattern or egg is used for scoring Very recently Saleh (25) has observed a marked increase in the NPU as determined in rats of Iranian diets when lysine was added Also Kaba (19) has shown that the amounts of lysine in the FAO provisional pattern is raised from 270 to 360 mg amino acid per g N Payne (23) has also argued in favour of raising the lysine content of the FAO provisional pattern to the same figure as Kaba

These facts would indicate that protein value of Iranian diets is limited by lysine and that it is highly possible that the diets consumed by the children at home were in fact limited by this amino acid

Our results on the usefulness of lysine added to bread are mainly negative (Tables 6 and 7) and indicate that school children that are offered a lysine limited meal do just as well (or even better) than those who receive the same meal to which lysine was added Explanations for this negative finding could be sought at the following levels

(a) Protein is not a limiting factor in the diets of our subjects and therefore increasing the biological availability of the nutrient will show no effect Surveys in Iran (8) have generally shown protein intakes to be only marginally adequate as an average and probably inadequate for a good number of the population The children showed heights weights and mid arm circumferences (Table 3) that run in the lower percentile for these anthropometric measurements in well nourished populations Both these facts could be interpreted to mean that in our subjects protein intake was inadequate

(*b*) The calorie intake rather than the protein is the limiting factor The meals served provided about 50% of the calorie allowances (Table 4) These might be short for children from Iranian villages because they are expected to do a considerable amount of work apart from attending school working in the fields shepherding carrying water walking to school etc In Iranian villages such as the ones where the trial was conducted there are extreme fluctuations in temperature (up to +45 in summer and down to −15 in winter) to which the children are much more exposed than in the industrialized countries a fact that can further increase their calorie needs If calories are the limiting factor then an increase in the quality of the protein can be expected to have little or no effect on growth

(*c*) The chemical scores do not actually reflect a lysine limitation in this age group We are aware that school children are not the best age group to study the effects of amino acid supplementation because of their slow growth rate Infants should be from this point of view much better Furthermore it is possible that lysine is more limiting i e the requirements for growth are greater at an earlier age (15) However the difficulties to be encountered in a field study that involves infants and pre school children would be great and far beyond that we could meet

Other explanations could be sought such as inadequacy of the parameters used in assessing the results insufficient time for observation imperfect control of the food intake non comparability of the groups that received the meals etc Still in our trial purposely designed

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vs after	*p*	0 001		N S	
No Lysine	*n*	81	81	75	75
	m	4 11	4 27	14 29	14 61
	S E M	0 054	0 077	0 111	0 134
Before	*t*	1 62		1 97	
vs after	*p*	N S		N S	
Control	*n*	69	69	67	67
	m	3 83	3 96	14 20	14 41
	S E M	0 504	0 411	1 07	1 18
Before	*t*	1 64		1 46	
vs after	*p*	N S		N S	

Mean ± S E M

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(b) The calorie intake, rather than the protein, is the limiting factor. The meals served provided about 50% of the calorie allowances (Table 4). These might be short for children from Iranian villages because they are expected to do a considerable amount of work apart from attending school: working in the fields, shepherding, carrying water, walking to school, etc. In Iranian villages such as the ones where the trial was conducted there are extreme fluctuations in temperature (up to +45° in summer and down to −15° in winter) to which the children are much more exposed than in the industrialized countries, a fact that can further increase their calorie needs. If calories are the limiting factor, then an increase in the quality of the protein can be expected to have little or no effect on growth.

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Other explanations could be sought, such as inadequacy of the parameters used in assessing the results, insufficient time for observation, imperfect control of the food intake, non comparability of the groups that received the meals, etc. Still in our trial purposely designed

to indicate the possible action of lysine supple mentation the addition of this amino acid to bread showed little or no effect On the other hand school meals themselves seem to have a very positive result This is evidenced by the differences in anthropometric measurements, especially weight between the children who received meals (L and NL groups) and the con trols who did not (Table 6) In this light school feeding programmes appear to be a more bene ficial measure to improve the nutritional condi tion of the children than does lysine enrich ment

SUMMARY

The effect of enrichment of bread with lysine was studied on a group of Iranian village chil dren receiving free school lunch over a period of 210 days Two groups received typical dishes which were purposely modified to make them limited by lysine one with enriched and the other with non enriched bread The third group did not receive lunch and served as con trol to assess the changes in the nutritional status of the former groups The food intake at the homes was not controlled

Markedly greater increases in weight and mid arm circumference were observed in groups which received the school lunch (with or without lysine) than in the control group Lysine enrichment showed no significant ef fect in improving the nutritional status except that children who received the amino acid sup-plementation exhibited significantly higher in crements in mid arm circumferences An in crement in height and weight was observed in children of non lysine group over those who received lysine which was significant in respect to height The latter group also showed a small but significant decrease in serum albumin

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APPENDIX 1

Composition of the premixes used in the enrichment of bread in th Lysine Programme (Iran)

1	Premix with vitamins and	kg
	minerals Wheat flour[1]	9 930
	Vitamins and minerals	0 825
2	Premix with vitamins minerals	
	and lysine Wheat flour	8 850
	Vitamins and mineral mixture	0 875
	Lysine hydrochloride	1 075
	Tartrazine	0 005

Preparation of enriched wheat flour

1	Wheat flour	48 0
	Vitamin and mineral premix	1 5
2	Wheat flour	48 0
	Vitamin mineral lysine premix	1 5

Composition of vitamin and mineral mixtures (prepared in Holland) (in kg)

Calcium carbonate	15 000
Vitamin A	0 1 4 (315 000 I U per g)
Riboflavin	0 030
Nicotinamide	0 100
Ascorbic acid (coated)	1 000
Iron sulphate (anhydrous)	0 190

Iranian wheat flour of about 95 extraction
Containing 77 lysine base Nederlandse Staats mijnen

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HUMAN GROWTH HORMONE THERAPY IN HYPOPITUITARISM DUE TO TUBERCULOUS MENINGITIS

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Endocrine complications due to tuberculous meningitis are not rare since the disease can affect important sites in the hypothalamus, the pituitary and the pituitary stalk (1–12, 14 16–18) Two females with short stature due to growth hormone deficiency following treatment of tuberculous meningitis are presented Both patients showed increases in growth velocity during administration of human growth hormone (HGH)

CASE REPORTS

Case I From 3 years of age this girl received for almost 2 years treatment with isoniazid and streptomycin for Tb meningitis Nine months after the initiation of therapy she developed polyuria and polydipsia The diagnosis of diabetes insipidus was then confirmed and Pitressin tannate 2 5 units every other day was added to her regimen with a significant decrease in diuresis She then measured 99 cm in height and weighed 15 5 kg Physical examination was unremarkable Skull X rays were within normal limits

Although the patient was under treatment with oral INH the disease recurred at 4 / years She was retreated initially with intramuscular and intrathecal streptomycin INH and PAS and then with a combination of pyrazinamide streptomycin and INH One month after inception of this treatment a calcification over the posterior clinoid processes was first noted Growth failure began to be manifest at 8 years From age 10 years when she measured 125 5 cm in height until 12 / years she had grown only 0 5 cm

An endocrine evaluation (Table 1) confirmed growth hormone deficiency Physical examination at this time disclosed her to be short and obese without any evidence of puberty Ophthalmological examination revealed a temporal scotoma of the left eye The patient was started on 2 mg *Human Growth Hormone* (KABI) intramuscularly three times weekly Administration of the hormone resulted in an 8 cm heigh increase in 13 months (Fig 1)

Case II is presently a 16 / year-old female At months of age she was hospitalized for Tb meningitis For 1 year she received streptomycin INH and PAS as well as prednisone with INH and PAS continued for a second year A temporary left hemiparesis was the only complication encountered during the acu phase of the disease She was thought to be doin well until 9 / years of age when her parents realize that she was very short At that time polyuria an polydipsia were first noted

She was first seen by us at 15 years of age Sh was 125 cm tall and relatively obese (37.5 kg) Sec ondary sexual development had not commenced bu the rest of the examination was unremarkable Endo crine evaluation summarized in Table – revealed GH ADH and LH deficiency There were no suprasella calcifications but 3 small calcifications were seen i the occipital area of the brain She was started o Pitressin tannate 3 units every 3 days This treatmen not only controlled her polyuria and polydipsia bu resulted in slightly improved growth a 3 cm heigh increase being recorded during 9 months Six month of treatment with HGH resulted in an increase o height from 1 8 cm to 136 1 cm (Fig 1)

DISCUSSION

It has been proved many years ago by the pathologists that tuberculous lesions of the pituitary region and the hypothalamus result in endocrine problems (3 4 13) A variety of endocrinopathies has been reported as sequelae of treated Tb meningitis (2 7–9 16 18) Has lam et al (5) reported inadequate GH secretion associated with diabetes insipidus low gonadotropins and intracranial calcifications in

Table 1 *Growth data and laboratory test results on Case 1 at 12¹/₂ years of age*

Height = 126 cm (<3rd perc)
Arm span = 178 cm
Weight = 38 5 kg (50)
Bone age = 11½ years

HGH assay

Minutes	Blood sugar (mg/100 ml)	HGH (ng/ml)
Insulin (0 15 U/kg iv) X2		
0	92	1
20	44	2
30	44	1
Gl ucagon		
0	88	0 5
5	80	0 5
15	112	0 5
30	120	0 5
60	72	0 5

PBI = 5 6 µg/100 ml

Urine

Total estrogens = 15 µg/100 ml
17 KS = 4 3 mg/24 hrs
17 HO = 4 4 mg/24 hrs

a 17 year-old male recovered from Tb meningitis While suprasellar calcifications are not a prerequisite for endocrine disturbances it has been calculated that 35 of all children treated for Tb meningitis will eventually

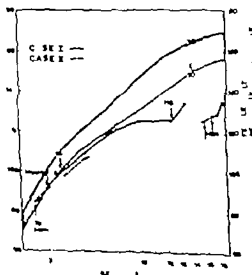


Fig 1 Growth chart showing both patients height growth pattern before and after treatment

Table 2 *Growth data and laboratory test results on Case 2 at 15 years of age*

Height = 125 cm
Arm span = 127 cm
Weight = 37 5 kg
Bone age = 10½ years

HGH assay (insulin 0 15 U/kg iv) × 2

Minutes	Blood glucose (mg/100 ml)	HGH (ng/ml)
0	64	3
15	32	2
30	16	1 5
45	12	1 5
60	24	2
75	32	1 5
90	40	1 5

Total serum thyroxine = 10 7 µg/100 ml
Urine LH = <25 µ/24 hrs
Urine total estrogens = <10 µg/24 hrs

SU — 4885

Day	17 KS	17 KSG (urine)	Plasma cortisol
1st Control	0	0	3 5 µg/100 ml at 8 a m.
2nd SU-4885 (250 6)	0	25 1 mg	
3rd Post SU-4885	2 9 mg	17 6 mg	
4th ACTH (gel 25 3)	4 6 mg	48 8 mg	

Urine S G = 1 005–1 008

develop intracranial calcifications after a few months or years (5) and most patients with hypothalamic calcifications following recovery from Tb meningitis develop endocrine complications These may become manifest immediately following onset of Tb meningitis or may occur several months or years later The endocrinopathies may result from primary hypothalamic and/or pituitary tuberculomata or can be secondary to thromboses or fibrosis with basal meningitis (5)

As expected in the 2 patients with growth hormone deficiency substitution therapy gave good results Both patients showed an abrupt increase in growth velocity in response to HGH 2 mg three times weekly This is the lowest recommended effective dose of HGH (15)

These cases stress the importance of long term supervision of children treated for Tb meningitis to detect the onset of the endocrine

sequelae which may follow after long periods of time Early detection of these and initiation of hormone substitution therapy can be of great help to these patients

SUMMARY

Lesions due to tuberculous meningitis resulting in endocrine dysfunction are not rare They usually cause inappropriate ADH secretion but occasionally other endocrinopathies related to the affected hypothalamic or pituitary regions are seen

Two female patients 13 $^1/_2$ and 16 $^1/$ years old treated in early childhood for tuberculous meningitis are presented Both were very short and had intracranial calcifications however the hypothalamic area was involved in only one of the patients Both had GH and ADH deficiency while one of them had gonadotropin deficiency as well Administration of HGH resulted in an adequate growth response

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SHORT COMMUNICATION

SEX CHROMOSOME ANOMALIES DETECTION AND FLUORESCENCE

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Fluorescence has proved of considerable as sistance in the detection of chromosome aber rations mainly the gonosomic ones as in the patient reported by Severi et al (1) The fol lowing case is presented as a further example of the use and limitations of the method

The patient was a 6-month-old boy referred for external genital abnormalities Chromo some analysis on peripheral blood cultures showed two cell lines (*a*) 45 chromosomes with 4 small acrocentrics (60%) and (*b*) 46 chromosomes with 4 small acrocentrics and a ring chromosome (40) (Fig 1) Autoradio graphy failed to reveal the presence of obvious late replicating chromosomes

Quinacrine fluorescence on metaphase plates gave ambiguous results Where the ring was contracted some preparations displayed dif fused and slightly above average fluorescence (Fig 2 *a*) while no specific fluorescence pat tern could be established in cases where the ring was uncoiled (Fig 2 *b*)

Examination of hair root and peripheral blood cells in interphase however led to the detection of a corpuscle of approximately 0 20 μ diameter (in 25 4 and 20 of the cells respectively) This was clearly distinguishable from the remainder of the nucleus by reason of its different fluorescence which was still slightly below the standard for Y chromo somes established in our laboratory (Fig 3)

The high frequency with which a fluorescent



Fig 1 Representative pro-metaphase from peripheral blood culture of the 46 chromosome cell line with a ring chromosome readily identifiable only in despiralized chromosome prepara tions

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CASE REPORT

LOWES SYNDROME

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Since Lowe described the special syndrome of organic aciduria decreased renal ammonia production hydrophthalmus and mental retardation in 1952 (6) about 150 similar cases have been reported in the literature The syndrome has also been described in Scandinavia (3 5 9 11 14) The most prominent features of Lowe's syndrome have been (i) Renal dysfunction aminoaciduria albuminuria intermittent glucosuria renal tubular acidosis and oligoammoniuria (ii) Ocular malformations cataract or glaucoma or both corneal opacities and myopic pupil (iii) Cerebral dysfunction mental psychomotor and growth retardation muscular hypotonia and hyporeflexia *Most of the* patients are boys but a *few* girls are described (4 12 13) It is thought that the disease is transmitted as a sex linked recessive trait (9 10)

CASE REPORT

Case 1 (H H) a male child born on 2nd May 1967 The parents were unmarried and unrelated Pregnancy and delivery were normal Birth weight was 3 010 g About 5 hours after delivery the child had repeated convulsions Apart from a relatively low blood glucose value (13 mg 100 ml) examination revealed nothing abnormal The blood glucose rose to 66 mg 100 ml but the child was hypotonic and did not feed well Ophthalmological examination 7 days after birth revealed striking hyperaemia of the iris on both sides myotic pupils with posterior synechiae and white opacities in both lenses Urinary examination showed proteinuria and haematuria Cystography showed a reflux to the ureter and pelvis on the left side

Two and a half months later the child was re examined because of convulsions He now showed obvious psychomotor retardation The muscles were hypotonic and there was hyporeflexia There were signs of serious renal damage The proteinuria had become pronounced and there was constant haematuria. Chromatographic examination showed a moderate aminoaciduria There was glucosuria Tubular phosphorus reabsorbtion test showed 55% Serum urea and creatinine values were normal The amino acid pattern of the serum was normal Serum calcium values were low from 7 3 mg/100 ml to 5 7 mg/100 ml There was hypokalaemia with values varying between 2 3 and 3 5 mEq/l The pH of capillary blood varied from 7 41 to 7.32 The child showed increasing oedema, ascites and hydrothorax presumably due to low serum protein levels (2 3–2.5 g/100 ml) During the last week he contracted a pulmonary infection and died on August 21 1967

Case 2 (H H) was female borne on 16th July 1968 by the same mother but allegedly of a different father Pregnancy and delivery were normal Birth weight was 3 100 g Soon after birth the following ophthalmological signs were observed The right eye was enlarged and prominent with a widened palpebral fissure (Fig 1) The corneal diameter was 12 mm The cornea was clear the anterior chamber a little shallow The iris appeared normal and the pupil was round with a fairly good reaction to light but with poor dilatation after mydriatics The lens showed a dense opacity at the posterior pole and in the temporal part of the cortex Unfortunately this opacity prevented a view of the fundus The left eye was smaller than normal the corneal diameter 8 mm The cornea was clear The anterior chamber looked deeper than normal The iris was strikingly hyperaemic The pupil was small with many posterior synechiae and vascular invasion The pupil could not be dilated There was a dense opacity in the nucleus of the lens and some dullness of the cortex The tension measured without anaesthesia was about the same on both sides 19–2 mmHg (Schiøtz)

Examination at the age of 1 month showed persistent proteinuria and haematuria aminoaciduria with





Fig 2 Quinacrine dihydrochloride stained metaphases showing a different fluorescence pattern of the ring chromosome if contracted (*a*) or despiralized (*b*)

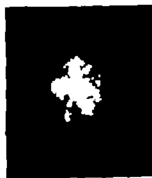


Fig 3 Interphase hair root cell with a fluorescent small corpuscle

corpuscle was observed in interphase cells indicates that it cannot be attributed to intensely fluorescent regions of chromosomes other than Y (2) Moreover the karyotype finding makes it clear that neither the Y chromosome nor its fluorescent segment is translocated

The presence of a Y chromosome in one of the two cell lines was confirmed by a subsequent diagnosis of mixed gonadal dysgenesis

Both parents displayed numerically and structurally normal chromosome complement while quinacrine staining showed the paternal Y chromosome to be normal with respect to shape size and specific fluorescence

To our knowledge this is the first case of Y ring chromosome so far reported in the literature In addition it may well be the only condition in which interphase fluorescence can resolve doubts engendered by the metaphase picture

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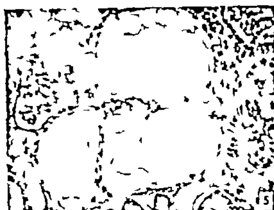


Fig 5 Kidney Dilated tubules



Fig 6 Kidney Small glomerulus Thickening of Bowmans capsule

lenses No calcium deposits were observed The angle of th anterior chamber and the canal of Schlemm appeared normal (Fig 4)

Kidneys

All the kidneys obtained from the patients were large and pale The kidneys from the male child weighed 58 and 61 g respectively totalling 119 g which is more than twice the averag kidney weight for a child of this weight. The kidneys of the female child weighed 33 and 36 g respectively totalling 68 g (normal values 50-52 g) The tubules were dilated with low atrophic epithelium (Fig 5) containing proteinaceous matter Some glomeruli were small with little blood in the capillaries (Fig 6) Adhesions were found in some places b tween th capillaries and Bowmans capsule as well as occasional thickening of the capsule (Fig 6)

Brain

The male child Gross examination revealed developmental deficiency in th posterior two-thirds of the corpus callosum The capsula externa was small and unevenly demarcated The cerebellum app ared normal There was no evidence of hydrocephalus Microscopical examination of the cortical layers revealed unsharp demarcation and poor differentiation of the neurons Ectopic cortical tissue was found in the marrow of the parietal and frontal regions Ectopic brain tissue was also present in the leptomeninges of the basal region The hippocampal region displayed irregular folding Th temporal horn was divided into two parts by a broad septum of glia tissue Irregular folding in the folia of the cerebellum was noted but the structure of the cortex was normal

COMMENTS

The two reported cases present the typical findings of the oculo-cerebro-renal syndrome of Lowe They present several interesting features especially from the genetic point of view Both had severe renal damage resulting in a fatal outcome as in the special variant described by McCance (8) The pathological anatomy described conforms well with previous reports (1 2) although few are available

The term "Lowe's syndrome probably includes more than one clinical entity The classical form of the disease is probably X linked recessive as for example in the four generation family reported by Pallisgaard & Goldschmidt (9) Since patients do not reproduce and no linkage with other X linked conditions has yet been established sex limited dominant inheritance cannot be excluded but is inherently improbable The mild ocular manifestations in heterozygotes in some families (10) are compatible with either mode of inheritance

There are also indications of one or more autosomal recessive forms of the disorder Matsuda et al (7) report a few cases which might well be autosomal recessive and biochemically distinct In these cases a defective bicarbonate reabsorption in the proximal renal tubules was documented as opposed to other cases of Lowe's syndrome with a probable distal renal tubular acidosis In addition several authors have described a more severe form which may occur in both sexes In the family of McCance



Fig 1 Female child with Lowe's syndrome

increased amounts of proline hydroxyproline glycine and phosphoethanolamine There were no abnormalities of the amino acid pattern of the serum Calcium values of the serum varied between 6 4 and 8 4 mg/100 ml phosphorus being about 5 6 ml/700 ml The pH of the capillary blood was 7 41–7 43 standard bicarb 24 35 mEq/l Tubular reabsorption test showed 75 Serum protein levels were about 3 5 g/100 ml Immuno electrophoresis showed low concentrations of gamma G and gamma A Chromosome examinations showed a normal female karyotype The child's general state deteriorated rapidly She became hypotonic with pronounced hyporeflexia She gradually displayed signs of psychomotor retardation She contracted several intercurrent infections and died on November 26 1968 probably due to a staphylococcal pneumonia

Pathologic Examinations

Pathological changes were observed in the eyes, kidneys and brain of both patients The lungs of the girl showed extensive inflammation

Eyes

Similar pathology was found in the eyes of both patients Retinal detachment was present although this could have been an artefact due to the histological preparation (Fig 2) The retina displayed irregular folding and numerous rosette formations (some of which contained hyaloid arteries at their centres) The layers affected were the outer corneous layer the rods and cones as well as the membrana limitans externa A few unconspicuous ganglionic cells were seen along with fibrosis and gliosis especially around blood vessels There was an increase of glial tissue in the optic nerve The cornea appeared normal The iris displayed hyperaemia and infiltration with white blood cells The pigment layer was attached to the lens in some places Cataract and nodular excrescences of the epithelium (Fig 3) had developed in all the







Fig 2 Longitudinal section of one eye
Fig 3 Lens Cataract and nodular excrescence
Fig 4 Camera anterior Canal of lemm

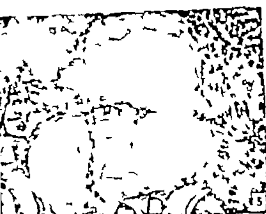


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Fig 4 Camera anterior Canal of Schlemm

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CASE REPORT

NEONATAL HYPERTHYROIDISM

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In each recorded instance of neonatal hyper thyroidism the mother was either hyperthyroid during pregnancy or had a previous history of thyrotoxicosis It is now considered that neo natal hyperthyroidism originates in the migra tion across the placenta of a 7S gamma globulin known as long acting thyroid stimu lator or LATS (5)

The purpose of this report is to describe one case of neonatal hyperthyroidism in a premature infant The essential features cor respond to those of classical Graves disease but in addition to the usual manifestations the child had heart failure and also exhibited a striking breast hypertrophy

CASE REPORT

Th patient is an infant female born to a 34 year old mother who has had two abortions and delivered one infant that died at 2 days of age

At age 18 the mother underwent partial thyroid ectomy for thyrotoxicosis with marked exophthalmos One year post surgery pretibial myxedema and club bing of her fingers developed

The mother was clinically euthyroid for 4 years prior to pregnancy and during pregnancy while re ceiving 0 mg l thyroxine daily No antithyroid drugs were given during pregnancy

Th child was delivered spontaneously at 34 weeks gestation Birth weight was 1 910 g. On admission to the pediatric ward at 4 hours of age th infant displayed sharp facial features but except for small size and prematurity had no abnormalities On physical examination the heart rate was normal (130 150) No h art murmur or signs of respiratory distress were noted

The child was placed in an incubator without suppl mental oxygen. At age 17 days th infant was transferred to a cot Moderate diarrhea developed on the tw nteth day *Salmonella oranienberg* was cul tur d from the stool Th same organism was isolated from oth r infants on the ward Loose stools persisted for 2 3 days The child became progressively more restless and irritable Despite a vigorous appetite th infant failed to gain weight and sh remained ex tremely hyperactive

Her eyes appeared rather bright and staring At the b ginning of the fourth week, periorbital edema d veloped accompanied by a pronounced bilateral increase in breast size (Fig 1) The heart rate in creas d to 180 per minute The liver enlarged to 2 5 cm below costal margin The child had no palpable enlargement of th thyroid gland

In the space of a few hours the condition deteriorated Feeding was periodically difficult the child b came markedly less responsive had inter mittent respiratory distress watery stools and vomit ing Sinus tachycardia, with a rate of 200 per minute prominent T waves and left axis deviation were seen on ECG A diagnosis of impending thyroid storm was made

Treatm nt with Lugol s solution and digitalis was



Fig 1 Infant at 24 days exhibiting breast enlarge ment

et al (8) two brothers were affected, but in the family of Svorc et al (13) one girl and that of Scholten (12) two sisters were affected This form is therefore probably also autosomal recessive

Our own family in which a mother had a severely affected son and a severely affected daughter allegedly by another man is not readily explained by either X linked or autosomal recessive or dominant inheritance X linked recessive and sex limited dominant inheritance are unlikely since the girl was severely affected Autosomal dominant inheritance is unlikely since the mother was unaffected Autosomal recessive inheritance would require that both fathers were heterozygotes This would be possible if in fact the same man was the father of both children, or the two fathers were related but otherwise is an unlikely event

SUMMARY

Two siblings, a boy and a girl with characteristic features of the oculo cerebro renal syndrome of Lowe are described In both cases the impaired renal function was a predominant feature of the disease, and both died at the age of 3–4 months Whereas the classical form of the disease probably is X linked recessive other clinical variants suggest different modes of inheritance, possibly autosomal recessive

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CASE REPORT

NEONATAL HYPERTHYROIDISM

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In each recorded instance of neonatal hyper thyroidism the mother was either hyperthyroid during pregnancy or had a previous history of thyrotoxicosis It is now considered that neo natal hyperthyroidism originates in the migra tion across the placenta of a 7S gamma globulin known as long acting thyroid stimu lator or LATS (5)

The purpose of this report is to describe one case of neonatal hyperthyroidism in a premature infant The essential features cor respond to those of classical Graves disease but in addition to the usual manifestations the child had heart failure and also exhibited a striking breast hypertrophy

CASE REPORT

Th patient is an infant female born to a 34 year-old mother who has had two abortions and delivered one infant that died at 2 days of age

At age 18 the mother underwent partial thyroid ectomy for thyrotoxicosis with marked exophthalmos One year post surgery pretibial myxedema and club-bing of her fingers developed

The mother was clinically euthyroid for 4 years prior to pregnancy and during pregnancy while re ceiving 0 mg / thyroxine daily No antithyroid drugs were given during pregnancy

Th child was delivered spontaneously at 34 weeks gestation Birth weight was 1 910 g On admission to the pediatric ward at 4 hours of age the infant displayed sharp facial features, but except for small size and prematurity had no abnormalities On physical examination the heart rate was normal (130-150) No heart murmur or signs of respiratory distress were noted

The child was placed in an incubator without suppl mental oxygen At age 17 days th infant was transferred to a cot Moderate diarrhea developed on the tw ntieth day *Salmonella oranienberg* was cul tur d from the stool Th same organism was isolated from oth r infants on the ward Loos stools persisted for 3 days The child became progressively more restless and irritable Despite a vigorous appetite the infant failed to gain weight and sh remained ex tremely hyperactive

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In the space of a few hours the condition deteriorated Feeding was periodically difficult the child b came markedly less responsive had inter mittent respiratory distress watery stools and vomit ing Sinus tachycardia with a rate of 200 per minute prominent T waves and left axis deviation were seen on ECG A diagnosis of impending thyroid storm was made

Treatm nt with Lugol s solution and digitalis was



Fig 1 Infant at 4 days exhibiting breast enlarge ment

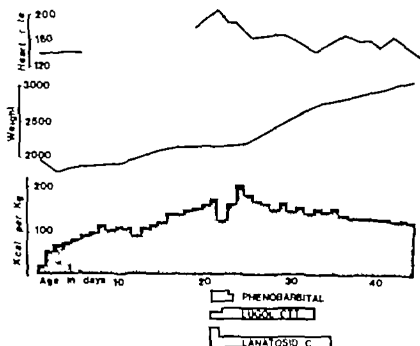


Fig 2 Growth in weight of the baby with curves representing caloric intake and heart rate

instituted Intravenous fluids were started and the child sedated with phenobarbital The infant responded dramatically during the following 6-8 hours with gradual improvement over the next few days The hyperactivity disappeared the diarrhea lessened and a weight gain was recorded The heart rate returned to 140-160 per minute the periorbital edema regressed and the breasts decreased in size Sedation was discontinued after 2 days Lugol's solution was continued for 11 days and digitalis for 14 days (Fig 2)

During the remaining hospitalization the child thrived Because of persistent cardiac enlargement and a moderately increased heart rate the child was redigitalized prior to discharge The heart rate subsequently returned to normal After 3 weeks digitalis was discontinued The heart gradually returned to normal size

Laboratory findings

Routine blood values were all normal Additional laboratory studies indicated normal urinalyses normal values of electrolytes cholesterol calcium and phosphorus

At delivery the PBI level in the mother was 20 µg/

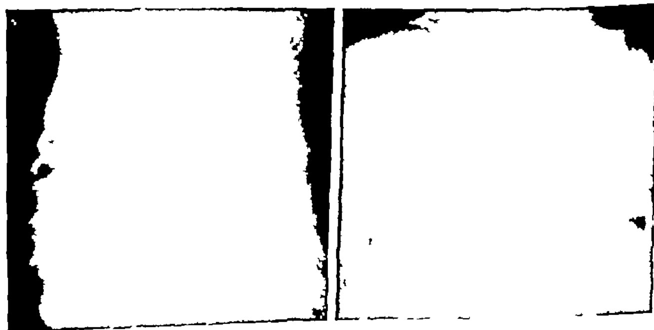


Fig 3 Anteroposterior radiograph of the chest at 4 and 11 weeks

100 ml The first PBI assay in the child obtained on the fifth day gave a value of 23 8 μg/100 ml On the 70th day after delivery the mother had a PBI of 14 6 and the child 27 8 μg/100 ml Serum thyroxine in the child measured after 25 days 36 days and 9 weeks was 15 1 12 4 and 7 5 μg/100 ml

Sera from the mother and the child failed to show either LATS or elevated TSH activity in serial measurements taken from the 25th day

In the mother tests for antibodies to the microsomal antigens of thyroid epithelium and to thyroglobulin proved negative Titre values obtained for gastric parietal cell antibodies 1 year before delivery and shortly after remained constant in the range of 16 32 determined by indirect immunofluorescent method using anti IgG-conjugate

In the child no thyroid antibodies could be detected 5 serial checks of gastric parietal cell antibodies remained unchanged with a titre of 8 from the 5th to the 11th week By the 15th week the titre dropped from 8 to 2 No further change was recorded by the 18th week when the last test was made The skeletal age of the child was estimated from X ray film of hand and wrist to be 6 months at a chronological age of 1 month and 14 months at age 3 / months according to Greulich and Pyle standards

DISCUSSION

From 1912 when White first described thyrotoxicosis in a newborn infant until 1971 about 40 cases have been reported (10 11) The course of neonatal thyrotoxicosis seems to follow the decay of LATS Kriss et al (9) demonstrated that LATS is a 7S gamma globulin McKenzie (6) has shown that LATS has autoantibody characteristics and the work of Miyai et al (8) indicated that LATS is synthesized in immuno-competent cells LATS is today considered to react with a TSH sensitive site in the thyroid cell The exact nature of the thyroid antigen however remains unclear

In the case reported the mild prodromal course followed by the child from birth may have been due to the fact that she was born 6 weeks prematurely and did not receive the terminal transfer of maternal gamma globulin Infection by Salmonella is believed to have induced the thyrotoxic crisis however it is unlikely that all symptoms observed were due to the Salmonella infection

Long acting thyroid stimulator has not been found in every instance of thyrotoxicosis In this case the mother had a previous history of Graves disease with marked exophthalmos clubbing of the fingers and pretibial myxedema all of which especially the last are good clinical pointers to the presence of high LATS levels (4 6) In spite of this neither the mother nor the child possessed detectable levels of LATS This was perhaps due to the relatively low sensitivity of the biological methods used for the assay of LATS In general biological methods are far less sensitive than immunological ones However so far no immunoassay for LATS is available

The increased incidence of autoantibodies against other organs in patients with thyrotoxicosis is well known The occurrence of gastric parietal cell antibodies in the mother therefore indirectly supports the view that autoimmune mechanisms are involved in her thyroid disease even though neither LATS nor antithyroglobulin nor antibodies against thyroid microsomes can be demonstrated in her serum The antibodies against gastric parietal cells in the infant s serum are most likely acquired by transplacental transfer but the elimination of the antibodies is more protracted than expected from the normal catabolism of immunoglobulin G

Whether hyperthyroidism can be the sole cause of heart disease has been disputed This case indicates that frank cardiac failure may occur in hyperthyroid neonates The cardiac decompensation disappeared when the child became euthyroid and the patient had sinus rhythm and normal heart sounds with no murmur there were no sequelae after recovery

Occasional reference has been made in the literature to breast enlargement associated with hyperthyroidism which receded after return to the euthyroid state Such observations imply that the hyperthyroid state may influence breast enlargement by altering the hormonal milieu The pathogenesis however remains obscure

The treatment of choice in the pregnant woman with thyrotoxicosis is still a matter of

debate (1 2, 7) But in the same context, it should be noted that recent researches on thyroxine derivatives in amniotic fluid indicate the possibility of documenting abnormal fetal thyroid function prenatally (3)

SUMMARY

Neonatal Graves disease with frank cardiac failure and pronounced bilateral breast enlargement, is described in a premature infant female

Thyrotoxicosis culminating rapidly in thyroid storm was successfully treated by administration of Lugol s solution digitalis and phenobarbital

The case is discussed in reference to the legitimacy of long acting thyroid stimulator (LATS) as the originator of neontal thyrotoxicosis

Although LATS could not be detected, the occurrence of gastric parietal cell antibodies in both mother and child yielded presumptive evidence for thyrotoxicosis as an autoimmune disease

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CASE REPORT

PRIMARY HYPERPARATHYROIDISM IN CHILDHOOD

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A diagnosis of primary hyperparathyroidism is seldom made in children In a survey of the literature till 1967 a total of 48 cases was collected (14) Since then 6 more cases have been reported (2, 3 4 6 12 16) two of these from Scandinavia (2 3) A review of the literature till 1970 is given by Bjernulf et al (3)

Primary hyperparathyroidism in childhood (PHPC) may be encountered both in infants and children but the disease seems most frequent in the pre adolescent and adolescent age group Including some doubtful cases, 12 of the reported patients are infants (1 4 6 7 14 18 19 20) 2 of these were brothers with symptoms starting in the neonatal period (9) PHPC is more common among males than females (ratio 3 2) In all the infants hyperplasia of one or more of the parathyroid glands has been found while parathyroid adenoma is the most frequent lesion in the older children Pronounced hypercalcemia and bone involvement are often seen in infants and younger children while renal lithiasis is more common in later childhood where generally the degree of hypercalcemia is less severe

The following case report is concerned with a 12 year-old boy in whom calcium metabolic studies led to a diagnosis of primary hyperparathyroidism and successful removal of a parathyroid adenoma

CASE HISTORY

N L K is a 12 year-old boy who had been in good health till the age of 10 and 11 when he was admitted 3 times because of abdominal pains and hematuria Urinary sediment showed a slight, intermittent hematuria but urinary culture intravenous urography cystoscopic examination of the bladder and renal function were normal Six months after the third admission the patient was readmitted with the same complaints Physical examination was normal but again hematuria was noted Radioisotopic renography gave evidence of a 1 ft sided partial ureteral obstruction which was confirmed by a repeated intravenous urography A few days later the patient had an episode with low back pains and hematuria, during which a renal stone was passed

Laboratory studies

Hematologic examination including hemoglobin erythrocyte leucocyte and thrombocyte count bleeding and clotting time and sedimentation rate were normal In the urinary sediment the number of erythrocytes varied within 0–130 Urinary cultures were normal

Serum calcium concentration and urinary calcium excretion were increased (Fig 1) Serum phosphate concentration was generally low and within the range 21 and 32 mg/l Serum alkaline phosphatases were normal The concentration of serum potassium and serum sodium were within normal limits

The renal function assessed by serum creatinine and 4 hour endogenous clearance of creatinine was normal

The electrocardiogram showed a typical WPW block

X rays of the skeleton teeth and lungs were normal

Eye examination (slit lamp) revealed no signs of band keratitis

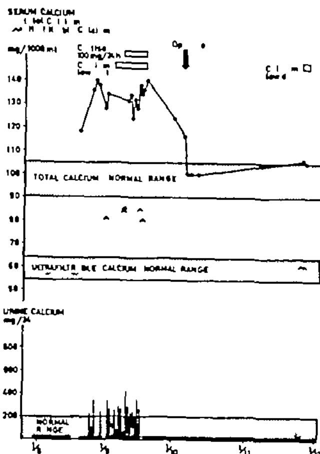


Fig 1 Concentration of total and ultrafiltrable calcium in serum and urinary excretion of calcium during admission Low calcium diet diet without milk cheese or vitamin supplements Cortisone test by use of cortisone 150 mg/70 kg body weight per day *Abscissa* date *Single* total serum calcium estimations were performed in the hospital routine laboratory by flame photometry *Paired* total and ultrafiltrable serum calcium estimations were performed in the hospital calcium metabolic laboratory using Halver s method (8)

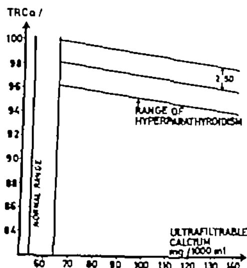


Fig 2 The tubular reabsorption of calcium (TRCa%) related to the concentration of ultrafiltrable calcium in serum in the patient *before* (O) *during* (●) administration of cortisone and *after* operation (×) The range of hyperparathyroidism has previously been published (21) and is reproduced by courtesy of Ib Transbøl

Further studies of calcium and phosphate metabolism and clinical course

In order to differentiate between primary hyperparathyroidism and a non parathyroid hypercalcemic condition further calcium and phosphate metabolic studies were carried out On a low calcium diet a determination was made of total and ultrafiltrable calcium concentration in serum serum phosphate concentration and urinary calcium and phosphate excretion (Table 1) From this and the concentration of creatinine in serum and urine calculation of the tubular reabsorption of calcium (TrCa) (21) the phosphate excretion index (PEI) (15) and the tubular reabsorption of phosphate (TRP) (7) was performed All these parameters including a cortisone test (5) (by use of 150 mg cortisone per 70 kg body weight/day) were indicative of primary hyperparathyroidism and 2 weeks later an explorative operation on the neck was performed and at the position of the right inferior parathyroid gland an adenoma weighing 780 mg was removed A freeze section microscopy confirmed the diagnosis and showed no signs of malignancy The postoperative course was uncomplicated and calcium and phosphate metabolism were normalized (Fig 1 2 and Table 1) At follow up in the outpatient clinic up to the present (one year after operation) the patient has been without symptoms, and serum calcium and serum phosphate concentrations have remained within the normal range Estimations of bone density by repeated bone scannings have indicated increasing mineral content of the skeleton since the operation

DISCUSSION

In reviewing the literature on PHPC it is remarkable that calcium and phosphate metabolic studies are few and in most cases the diagnosis has only been based on estimations of serum concentrations of calcium and phosphate and histologic examination of the parathyroid glands removed at operation or autopsy In childhood, hypercalcemia may be a cardinal symptom in other conditions of disease e g vitamin D intoxication sarcoidosis and congenital idiopathic hypercalcemia Therefore in order to avoid unnecessary neck explorations any case of unexplained hypercalcemia should be subjected to further metabolic studies, before operation is undertaken

Table 1 *Serum calcium and phosphate concentration and the renal handling of calcium and phosphate before and during administration of cortisone and after the removal of a parathyroid adenoma*

	Serum			Urine				
	Calcium (mg/1000 ml)		Phosphate (mg/1000 ml)	Calcium (mg/24 h)	Phosphate (mg/24 h)	TRCa	24 h PEI[b]	TRP
	Total	Ultrafiltr						
	(II)	(II)		(11)			−0 09–	> 85
Normal range	90–103	54–64	40–70	40 200			+0 09	
The last 2 days before cortisone	139 3 140 1	85 9 86 4	24 24	93 284	250 430	97 0 96 1	+0 22 +0 15	72 3 78 7
Cortisone day 8+9	141 7 140 5	85 3 81 1	28 27	347 265	490 565	96 3 96 6	+0 08 +0 09	83 8 82 9
7 weeks *after* operation	105 1 105 9	61 3 61 2	46 48	42 58	370 557	99 1 99 3	0 14 0 16	89 3 91 8

TRCa = the tubular re absorption of calcium expressed as per cent of the filtered load of calcium
[b] 24-h PEI = the 24-hour phosphate excretion index
TRP = the tubular re-absorption of phosphate expressed as per cent of the filtered load of phosphate

Measurement of the TRCa PEI TRP % and cortisone test which have been employed in the present case can all be of value in the differential diagnosis In adults determination of the TRCa is useful irrespective of the degree of renal insufficiency at least at clearances of creatinine ranging from 13–177 ml/min (21) The phosphate excretion tests offer no such distinction in states of hypercalcemia especially when renal insufficiency supervenes (21)

In the present case all the metabolic studies pointed to a diagnosis of primary hyperparathyroidism The patient had a cortisone resistant hypercalcemia with a moderate elevation of both total and ultrafiltrable calcium The urinary calcium excretion was moderately increased and the TRCa was high and within the range of hyperparathyroidism The serum phosphate concentration was low and the high PEI and low TRP% were consistent with an increased renal phosphate excretion The postoperative change in TRCa % PEI and TRP indicated body conservation of calcium and phosphate resulting in rebuilding of the skeleton This was confirmed by bone scanning even though halisteresis was never demonstrable on roentgenograms

In adults the frequency of primary hyperparathyroidism is rather high An incidence of 13 cases of primary hyperparathyroidism out of 3 895 admissions (including re admissions) is found in a recent report from a medical department where routine serum calcium estimations were performed in all patients (10) Twenty five per cent of the patients with primary hyperparathyroidism are between 15 and 35 years old (13) Considering the frequency and the age distribution of the disease in adults it seems most likely that many of the cases diagnosed in early adulthood may have started in childhood Without doubt the disease is underdiagnosed in the pediatric age group and the 54 cases of PHPC reported in the current literature do not seem to give a realistic measure of the incidence With the heterogenous clinical picture of the disease and the excellent results of early treatment in mind liberal estimations of serum calcium concentration should be done in all uncertain states of disease in children

SUMMARY

A case of primary hyperparathyroidism in a 12 year-old boy with renal lithiasis is reported Laboratory investigations showed a cortisone resistant hypercalcemia and a low serum phos

phate concentration The renal handling of calcium and phosphate too was indicative of primary hyperparathyroidism At the following operation a parathyroid adenoma was removed The importance of estimating the serum calcium concentration in all uncertain states of disease in children is stressed since primary hyperparathyroidism is probably underdiagnosed in the pediatric age group

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CASE REPORT

CLINICAL MANIFESTATIONS OF LETTERER SIWE DISEASE IN THE NEONATAL PERIOD

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Letterer Siwe disease is a disseminated macrophage proliferation involving all organs containing a reticuloendothelial component Although this disease is a rare clinical entity in the newborn period an early diagnosis is possible as the clinical findings are dominated by characteristic skin lesions In some cases of Letterer Siwe disease diagnosed in later infancy a transitory but non specific rash has been observed in the neonatal period In this report the clinical manifestations of congenital Letterer Siwe disease in a newborn baby are presented and some aspect of diagnosis and therapy are discussed

CASE HISTORY

A boy first child to a 25 year-old healthy mother was born in the 43rd week of gestation with birth weight 3 580 g No history of hereditary disease At birth there were wide spread bluish red maculo-papules varying in size from a few millimetres to about 1 5 cm On the ventral side of the thorax and on the left lower eyelid firm elevated discoid nodules 3 cm in size were observed (Fig 1) Several of the lesions became ulcerated and covered with a black eschar within the first 24 hours of life Enlarged lymph nodes were noted in the neck and groin

Laboratory examinations

Routine blood examinations including haemoglobin white blood cell differential counting and platelet count as well as bleeding and coagulation times were normal Initial X ray of the chest was normal Serological tests for syphilis listeria monocytogenes toxoplasmosis and cytomegalovirus infection were normal in the mother and in the baby Cytomegalic inclusion bodies were not found in the epithelial cells in the urine Immunoelectrophoresis on the 3rd day of life showed IgG 570 mg/100 ml IgA 1 mg/100 ml IgM 9 mg/100 ml i e normal values Virus and bacteria cultures from specimens of faeces lymphoglandular punctures throat washings and from the skin lesions were negative A Coxsackie B5 virus was isolated from the urine

Bone marrow aspirate (crista iliaca) appeared normal

Biopsy findings

Fine needle biopsy from an inguinal lymph node obtained on the 1st day of life gave an aspirate dominated by mononuclear cells with ample cytoplasm and rounded slightly polymorphous nuclei Fairly numerous mitoses were seen The cytological picture was compatible with that of a reticuloendothelioma

Cutaneous biopsy (of nodules) on the 2nd day of life showed a histiocytic infiltration in the dermis with tendency to arrangement round vessels permeation into and ulceration of the epidermis

Microscopic examination of the placenta showed non-specific inflammatory changes but was otherwise normal

Course

During the first week of life the maculopapular skin lesions remained almost stationary Physical examination on the 7th day of life showed besides the cutaneous lesions enlargement of the lymph nodes but no hepatosplenomegaly Routine blood examinations revealed a certain degree of anaemia but were otherwise normal

During the following days the disease took a fulminant course there was a progressive deterioration with pallor irritability and feeding difficulties



Fig 1 Widely distributed blue red maculopapules and an elevated discoid nodule on the left lower eyelid on the first day of life

Bacteriological cultures from the skin lesions showed plasmacoagulating *Staphylococcus aureus* Treatment with antimetabolites was planned but there was a rapid decline and the baby died on the 18th day of life

Autopsy findings

In addition to the generalised cutaneous manifestations the autopsy examination revealed multiple macroscopically demarcated small nodules in the thymus the liver the spleen the lungs and in the urinary bladder A generalised lymphadenopathy was also found No bone lesions were seen in the spine

Microscopical examination

Th above mentioned disseminated lesions were composed of large cells with rich eosinophilic cytoplasm and polymorphous vesicular nuclei with prominent nucleoli The structure of the lymph nodes and the thymus was almost completely obliterated by diffuse and nodular histiocyte proliferation The same type of histiocytes was found in the lung parenchyma infiltrating the alveoli and the alveolar septa

DISCUSSION

Until now only five cases of Letterer Siwe disease diagnosed in the neonatal period have been reported in the literature (1 3 4 7 9) The clinical findings in these cases were dominated by the same cutaneous manifestations as seen in our case i e a few elevated ulcerated nodules and a disseminated bluish red maculopapular rash Non specific skin lesions such as seborrhoic dermatitis later disappearing or slowly deteriorating have been noted at birth in some infants, who did not develop subsequent signs and symptoms of Letterer Siwe disease until later in infancy (1 4 5 7 10 11) In order to obtain an early diagnosis, a skin biopsy must be carried out within the first days of life Fine needle biopsy should be performed when lymphoglandular enlargement is noted

Despite the occurrence of the 'typical cutaneous manifestations of congenital Letterer Siwe disease several other conditions should be considered in the differential diagnosis e g congenital leukaemia, mastocytosis and transplacental infections including congenital syphilis listeria monocytogenes rubella fetopathy herpes simplex infection variola and varicella

The prognosis of Letterer Siwe disease is closely related to the age of the infant and to the degree of dissemination of reticuloendotheliosis when the diagnosis is determined During the first six months of life the prognosis has been extremely poor with a mortality rate of at least 70 (6 8 9) The two most commonly affected organ systems are the skin and the skeleton In the case reported here there was a pronounced reticuloendothelial dissemination in several organs including skin, lungs thymus liver spleen and lymph nodes

Spontaneous recovery has been reported in patients not so extensively affected and remission has followed treatment with antimetabolites antibiotics steroids radiation or alkylating agents given either separately or in combination (2 9 12 13) One newborn infant

with congenital Letterer Siwe disease has also been treated with vincristine sulphate and corticosteroids with gratifying results (7)

SUMMARY

The clinical manifestations of congenital Letterer Siwe disease in a newborn baby have been described Diagnostic procedures including biopsy of skin lesions and fine needle aspiration of existing lymph nodes should be performed without delay as early treatment may induce remission

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

DANISH PAEDIATRIC SOCIETY

Meeting January 14 1972

Hanne Bærentsen *Hypoglycaemia A case of hyperinsulinism* Published in *Acta Paediat Scand 62* 207 1973

E Damgaard Andersen, J Ramsøe Jacobsen E Sandøe A Wennevold & J Videbæk *Paroxysmal tachycardia in infancy and childhood* To be published in *Acta Paediat Scand 62* 1973

P O Schiøtz & J Helweg Larsen *A case for diagnosis*

The case history of a girl aged 2 years was presented There was no known familial predisposition She was the first of two children Pregnancy delivery and birth weight were normal and no abnormalities occurred in the neonatal period The patient presented the following symptoms from birth Failure to thrive retarded psychomotor development, retarded skeletal development, severe oedema of the lower limbs, moderate hepatomegaly and moderate granulocytopenia with greatly lowered resistance to infections Laboratory investigations included normal chromosome findings and the bone marrow puncture revealed inhibition of leukocyte maturation Diagnosis Infantile genetic agranulocytosis?

Meeting January 28 1972

Marshall Klaus *Mother to infant attachment —significance of early postpartum period*

Studies of a wide range of mammalian mothers and newborns have shown that each species exhibits sequences of maternal behavior around the time of delivery and during the first days and months of life Interference with these patterns may result in undesirable even catastrophic, effects on the young Controlled studies in the human mother have suggested that present hospital practices which separate the premature sick and even full term infants from their mothers may produce a severe iatrogenic disease in the human mother and may result in aberrant behavior toward her infant months or years after the birth These and other observations suggest that major alterations of maternal behavior may be brought about by a rather simple modification of hospital practices affecting the mother–infant dyad and indicate that the extent of close physical contact in the early hours and days of life may have a far more powerful formative and patterning effect on the mother than has been previously appreciated

Meeting February 11 1972

PRIMARY IMMUNE DEFECTS

Viggo Faber *Primary immune defects*
(not received)

Klaus Jensen *Isolation in germ free environments*
(not received)

Chr Koch Kirsten Henriksen & Frede Juhl *Bone marrow transplantation of an infant with severe combined immune defect*

A five month-old female infant was referred with the diagnosis of "Severe Combined Immunodeficiency Syndrome

The severe clinical condition prompted an attempt at transplantation of allogenic bone marrow No histocompatible sibling was available as donor but after HL-A typing and mixed lymphocyte culture studies the father was chosen as the most suitable donor Circumvention of the anticipated graft versus host reaction was attempted by fractionation of the bone marrow

An initial transplant with 2×10^{6} nucleate bone marrow cells/kg body weight did not insure a satisfactory "take of the graft Increasing the number 4 weeks later to 5×10^{6} resulted in a take of the graft Partial reconstitution of cellular immunity was evidenced by a strongly positive reaction to stimulation with Phytohemagglutinin 37 days after the second transplant Symptoms of graft versus host disease appeared on the 27th day and persisted in moderate to severe form Isolation in sterile environment on a laminar air flow bench and extensive bacterial de-contamination proved highly effective since no exogenous microorganisms contaminated the child during the entire period of isolation A strain of *E coli* was present in inflammatory lesions upon admittance however and this strain reappeared on several occasions In connection with the graft versus host disease and bone marrow depression widespread *E coli* dissemination occurred The child succumbed 71 days after the second transplant to overwhelming *E coli* septicemia

It is concluded that fractionation of bone marrow prior to transplantation in non histocompatible cases may postpone onset and possibly decrease the severity of graft versus host disease Furthermore successful bacterial decontamination seems to be of primary importance prior to and during periods of graft versus host disease

Kirsten Henriksen Frede Juhl & Christian Koch *Continuous fluid and electrolyte therapy for 3 1/2 months*

A case of prolonged diarrhoea in an infant is described The infant suffered in addition from a severe combined immune defect of such severity that fluid administration corresponding to 33 of the body weight proved necessary for months

The diarrhoea started along with infection and continued on account of malnutrition and massive oral neomycin administration The graft versus host reaction also contributed to some extent to the severity of the diarrhoea

It is necessary in view of alimentary function to establish the diagnosis of severe combined immune defect rapidly Intravenous catheters should be avoided as far as possible until decontamination has occurred It is maintained further that recontamination should not be undertaken in connection with a graft versus host reaction

Bo Dupont Vagn Andersen et al *Diagnosis of severe combined immune defect by lymphocyte studies in vitro*
(not received)

Meeting February 25 1972

H T Lund & J Jacobsen *Influence of phototherapy on unconjugated bilirubin in duodenal bile of newborn infants with hyperbilirubinemia* Published in *Acta Paediat Scand 61* 693 1972

C Joh Ingomar & I Klebe *The acid–base ratio in capillary blood in neonates The influence of placental transfusion* Published in *Acta Paediat Scand 62* 21 1973

J Christoffersen T Marner & E Raabo *LDH isoenzymes in the serum in children illustrated by simultaneous capillary and venous samples*

The concentration of total LDH in the serum in infants is approximately double that in adults Adult values are not attained until about puberty During the first months of life the LDH isoenzyme pattern shows relatively low values for isoenzyme no I In paediatrics isoenzyme determinations are of greatest value in the diagnosis of muscular dystrophy and sphingolipidosis

By simultaneous withdrawal of capillary and venous blood from 41 normal infants significantly lower values for isoenzyme no I were found in the capillary blood than in venous blood while the other four isoenzymes had significantly higher values in capillary blood This difference may be attributed to admixing of LDH isoenzymes from the skin and subcutaneous tissues in sampling the capillary blood Insufficient correlation between simultaneous capillary and venous values in pairs was shown Assessment of a LDH value must thus be undertaken by comparison with a normal figure obtained by the same sampling method

K Thomsen S Sparrevohn & J Haahr *Renal involvement in Schonlein Henoch s purpura*

In a material from the paediatric departments in Greater Copenhagen from a 10 year period a total of 203 children with Schonlein Henoch s purpura were encountered Of these 60 (30 •) had haematuria during the acute phase of the disease Follow up investigation 1–10 years later of 50 of the children with primary renal involvement revealed that one child had died from acute proliferative glomerulonephritis and 6 others had developed chronic glomerulonephritis which had been verified by renal biopsy On the other hand, 30 children in whom no primary renal involvement occurred were all healthy on follow up investigation

Chronic glomerulonephritis thus develops in approximately 3 • of children with Schonlein Henoch s purpura (and in approximately 14% of the children who have haematuria at the commencement of the illness)

DISCUSSION

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The 2 patients with severe renal involvement were treated with prednisone Endoxan and Imurel without effect on the renal function either

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A boy aged 6 years was admitted on account of pain in the nuchal region and back and headache

Pronounced neck rigidity and tenderness between the scapulae was found The temperature was slightly raised but the general condition was good

Radiographic examination revealed calcification in the intervertebral disc between thoracic vertebrae XI and XII and a skeletal age of approximately 3 years

Examination of the cerebro spinal fluid revealed normal conditions ESR 46 m

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The symptoms disappeared without treatment after hospitalization for a week. On follow up examination 7 months later the patient remained symptom free but the calcification was unchanged.

No etiological explanation could be found.

Meeting March 10 1972

F Utzon & F Brandrup *A blue baby*

To be published in *Acta Paediat Scand* 62 1973

H Verder Kirsten Stehr Johansen & K Engbek *The diagnosis of Hirschsprung's disease by means of anal sphincter tonometry*

Published in *Acta Paediat Scand* 62 59 1973

Ragnhild Dissing & A Villumsen *Renal contusions in children*

During the period 1962–71 a total of 17 children were treated for renal contusions. Nearly all of the children were aged between 6 and 12 years. Eleven of the children had macroscopic and 6 had microscopic haematuria. The commonest urographic findings were spastic pyelograms (7 patients). Five patients had normal urograms. Two patients were submitted to operation on account of rupture of the spleen and 5 were submitted to operation on account of suspected intraperitoneal lesion with normal operative findings.

Treatment was conservative i e rest in bed until the microscopic haematuria had disappeared. This occurred as a rule in the course of 2 weeks. None of the patients were submitted to emergency operation on account of the renal lesion but secondary correction was undertaken in a patient who developed hydronephrosis 6 months after the trauma.

The children were followed up every 6 to 12 months with urography as long as pathological findings were encountered which might be considered to influence the future functioning of the kidney. The blood pressure was followed up every 6 to 12 months until adult life. None of the patients showed signs of hypertension. Four patients still had abnormal urograms on follow up examination.

Conclusions 1) In this material renal contusions in children were successfully treated conservatively 2) Intravenous urography should be undertaken as rapidly as possible after the trauma. Employment of a relatively large quantity of the radio-opaque agent is recommended for this 3) if no excretion is found on urography arteriography should be performed and 4) patients should be followed up in view of late complications particularly secondary hydronephrosis atrophy of the kidney and hypertension.

Ingrid Thorn *Phenytoin allergy*

A girl had at the age of 4 1/ years had prolonged generalized convulsions in association with pyrexia. Nine months later another bout of prolonged convulsions occurred.

Treatment with phenytoin was commenced (7 mg per kg). Twelve days later the patient became pyrexial in her home and 2 days later a generalized finely punctate exanthema developed. Both of these symptoms disappeared on withdrawal of phenytoin.

One month later the patient received 25 mg phenytoin and developed a subfebrile temperature and flushing of the face. Two hours later 25 mg amidryl was administered. Half an hour later she developed headache nausea malaise and progressive clouding of consciousness accompanied by generalized tonic/clonic convulsions. Stesolid was administered intravenously in a dosage corresponding to over 1/4 mg per kg to some effect. The general condition had been dominated by slight cyanosis which disappeared on administration of oxygen and superficial irregular respiration with a tendency to apnoea which could however be managed by light manual artificial respiration which proved necessary during the subsequent

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of these treatment with sulphonamides proved necessary. In the remaining patients the bacteriuria disappeared spontaneously after withdrawal of ampicillin.

Nine out of 37 children treated with penicillin developed bacteriuria. Only 2 of these had symptoms and in all of them the bacteriuria disappeared spontaneously. The incidence of bacteriuria was significantly higher in children treated with ampicillin than in those treated with penicillin but only in children under the age of 2 years.

The possibility of haematogenic infection from abnormal intestinal flora resulting from employment of broad spectrum antibiotics is discussed. It is concluded that ampicillin therapy in children under the age of 2 years should be followed up by control examination of the urine 2 or 3 weeks after cessation of the treatment and that ampicillin is not advisable as the standard treatment in this age group.

O. Steinicke: *Pyloric stenosis once more*

A newly born male infant had blood containing vomits during the first day of life. At the age of 12 days radiographic examination of the stomach was undertaken in view of hiatus hernia. This investigation revealed normal conditions and in particular normal prepyloric antrum.

At the age of 25 days the infant developed classical symptoms of pyloric stenosis and the diagnosis was verified by radiography on the 29th day of life.

These findings support the theory that degeneration of the intramural ganglion cells in the prepyloric antrum is secondary but that the symptoms do not commence until these are present.

H. Lerum: *A case of Wilson's disease treated with penicillamine*

A girl aged 12 years was admitted after suffering from recurrent jaundice for 4 months. Apart from some loss of appetite and occasional fever attacks the general condition had been relatively uninfluenced and this state of affairs persisted on admission. The urine had been dark but the faeces were not pale. Liver tests and electrophoresis revealed a cirrhotic pattern and juvenile cirrhosis was suspected. This suspicion was corroborated by the findings at liver biopsy which showed a histological picture of chronic active hepatitis.

A course of prednisone therapy was commenced but no effects were observed on the above mentioned laboratory findings after 1 month. The general condition remained relatively unaffected. The diagnosis was taken up for revision and the relevant laboratory tests for Wilson's disease (serum copper, ceruloplasmin and 24 hour excretion of copper in the urine) were performed. These revealed a pattern significant for Wilson's disease and ophthalmological examination revealed the pathological Kayser Fleischer ring in the limbus corneae.

Treatment with dimethylcystein was then initiated. This has been the best therapeutic agent for this disease since 1955. The anticipated increase in cupruresis resulted and after treatment for 3 months the results of liver tests and electrophoresis were normalized. The patient has since been followed up as an out patient. She is still well and the laboratory findings are normal. Treatment did not have any side effects.

The etiology of the condition is reviewed together with the pathogenesis and clinical features. Finally the main features in the presumed mode of action of dimethylcystein are outlined.

K. Rasmussen: *Specific screening for organic acids in blood and urine in children*

A gas-chromatographic mass spectrometric screening system has been evolved for identification and quantitative determination of organic acids and amino acid conjugates, e.g. acylglycines in the blood and urine. Deter

2–3 hours The patient gradually regained consciousness she was somewhat confused at first The blood pressure was 170/70 at the commencement of the condition but fell rapidly to 140/60 but did not return to normal until after 48 hours There was no evidence of eosinophilia One month later the patient had another grand mal seizure and phenemal therapy was instituted and tolerated

It is considered probable that the dramatic course in addition to being an allergic reaction to phenytoin may have been caused by the central stimulating effect of the antihistamine which is distinct particularly in patients with focal cerebral changes

Renewed phenytoin therapy after an initial phenytoin exanthema is contra indicated particularly if this is accompanied by pyrexia Similarly the employment of antihistamine preparations in epileptic patients is warned against

P A Krasilnikoff & K Egeblad *Bronchial foreign bodies in children*

(not received)

Meeting April 7 1972

P Geertinger *Sudden unexpected death in infancy*

Meeting May 13 1972

A Lindahl Olsen & P Roslyng *Polyradiculitis in childhood*

Fl Holck *A case of myotonia congenita*

Fl Jørgensen *A case for diagnosis—Mercury intoxication?*

A boy of 26 months was admitted with acute gastroenteritis and jaundice after illness in his home for 3 days On admission his general condition was poor he was dehydrated slightly jaundiced and had slightly elevated enzyme levels In addition he had conjunctivitis, stomatitis and skin changes localized to the distal phalanges of all the fingers and toes which were slightly swollen and showed bluish red discolouration In addition red erythema was observed on the palms of the hands Apart from the raised enzyme levels and bilirubin the other laboratory findings were normal

The jaundice disappeared after a week but the enzyme levels remained raised The enteritis persisted for the next month despite intensive parenteral and later, dietetic therapy Although energetic clinical and laboratory investigations were undertaken no explanation could be found for the patient's poor general condition which persisted for a total of 2 months After this the patient began to recover The skin changes on the hands and feet regressed and all of the finger and toenails were shed After 3 months recovery was complete

At this time the urine was investigated for the mercury content which was found to be raised to 100 μg/l (normal 0–50 μg/l) Control examination 1 month later showed the mercury content of the urine to be 3 μg/l The enzyme levels were still slightly raised

Klaus Bendtzen & A Lindahl Olsen *Ampicillin induced bacteriuria in children*

82 children aged from 1 month to 15 years and with previously healthy urinary tracts were treated with ampicillin or penicillin respectively on account of infections with presumably sensitive bacteria Out of 45 patients treated with ampicillin 24 developed significant bacteriuria with ampicillin resistent bacteria particularly Klebsiella and Proteus strains 67 % of these were asymptomatic while the remainder developed dysuria pollakisuria, secondary pyrexia and/or pyuria and in half

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Fl Holck *A case of myotonia congenita*

Fl Jørgensen *A case for diagnosis—Mercury intoxication?*

A boy of 26 months was admitted with acute gastroenteritis and jaundice after illness in his home for 3 days On admission his general condition was poor he was dehydrated slightly jaundiced and had slightly elevated enzyme levels In addition he had conjunctivitis, stomatitis and skin changes localized to the distal phalanges of all the fingers and toes which were slightly swollen and showed bluish red discolouration In addition red erythema was observed on the palms of the hands Apart from the raised enzyme levels and bilirubin the other laboratory findings were normal

The jaundice disappeared after a week but the enzyme levels remained raised The enteritis persisted for the next month despite intensive parenteral and, later dietetic therapy Although energetic clinical and laboratory investigations were undertaken, no explanation could be found for the patient s poor general condition which persisted for a total of 2 months After this the patient began to recover The skin changes on the hands and feet regressed and all of the finger and toenails were shed After 3 months recovery was complete

At this time the urine was investigated for the mercury content which was found to be raised to 100 $\mu g/l$ (normal 0–50 $\mu g/l$) Control examination 1 month later showed the mercury content of the urine to be 3 $\mu g/l$ The enzyme levels were still slightly raised

Klaus Bendtzen & A Lindahl Olsen *Ampicillin induced bacteriuria in children*

82 children aged from 1 month to 15 years and with previously healthy urinary tracts were treated with ampicillin or penicillin respectively on account of infections with presumably sensitive bacteria Out of 45 patients treated with ampicillin 24 developed significant bacteriuria with ampicillin resistent bacteria, particularly Klebsiella and Proteus strains 67% of these were asymptomatic while the remainder developed dysuria pollakisuria, secondary pyrexia and/or pyuria and in half

NEW BOOKS RECEIVED

Evaluation of food additives World Health Organization Technical Report Series No 488 Geneva 1972 40 pp

H Begemann & J Rastetter *Atlas der klinischen Hamatologie* 2nd ed 324 pp illus Springer Verlag Berlin Heidelberg New York 1972 DM 248 —

H Bickel F P Hudson & L I Woolf (eds) *Phenylketonuria and some other inborn errors of amino acid metabolism* 336 pp illus Georg Thieme Verlag Stuttgart 1971 DM 88 —

A W Root *Human pituitary growth hormone* 259 pp illus Charles C Thomas Publisher Springfield 1972 $10 50

Th Baumann & R Frey (eds) *Epilepsie im Kindesalter* 2nd ed Padiatrische Fortbildungskurse fur die Praxis 140 pp illus S Karger Basel 1972 sFr 39 —

W Hamilton *Clinical paediatric endocrinology* Postgraduate Paediatric Series 209 pp illus Butterworths London 1972 £5 80

W Isler *Acute hemiplegias and hemisyndromes in childhood* Clinics in Developmental Medicine Nos 41-4 314 pp illus Spastics International Medical Publications William Heinemann Medical Books Ltd London 1971 £4 80

Peptide transport in bacteria and mammalian gut Ciba Foundation Symposium 161 pp illus Elsevier Excerpta Medica North Holland Assoc Scientific Publishers Amsterdam 1972 D Fl 20 —

B Rosm & E Gutther *Gehorstorungen beim Kind* 99 pp S Karger AG Basel 1972 sFr 33 —

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Kenneth R Bzoch & R League *Assessing language skills in infancy* 56 pp illus The Tree of Life Press Gainesville 1972 3rd printing $9 00

mination of non amino acids has not only revealed hitherto unknown inborn errors of metabolism but also abnormal intermediate metabolites in already known metabolic diseases This has resulted both in definition of primary metabolic block and has rendered possible a single but specific diagnostic investigation One example is the heterogenic syndrome described by Nyhan ketotic hyperglycinaemia" which can now be subdivided into three well defined errors in the organic acid metabolism Analysis for seondary *metabolites is the only reliable possibility* of revealing the diagnosis in patients in spontaneous remission (e g determination of propionyl tiglyl and isovaleryl glycine in the urine in the corresponding acidaemias) Analyses of this type can also reveal whether a dietetic treatment is optimal (e g tiglinic acid excretion in propionic acidaemia)

At present, at least 16 well defined organic acidaemias or acidurias are known The majority of the patients have intermittent ketoacidotic episodes, most frequently in the neonatal period In others, failure to thrive or mental retardation are cardinal findings

Knud E Petersen

NEW BOOKS RECEIVED

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Acta Pædiat Scand 62 332 1973

ANNOUNCEMENTS

MEDITERRANEAN AND MIDDLE EASTERN PAEDIATRIC CONGRESS

The Ninth Mediterranean and Middle Eastern Paediatric Congress will be held in Ankara Turkey from August 31 to September 3 1973

Themes

Social paediatrics nephrology immunology genetics and radiology Free papers accepted

Registration fees

$45 regular members $30 associate members Bank drafts to be sent to the Congress bank account No 865289 3 Osmanli Bankasi Merkez Subesi Ankara Application forms obtainable from Congress Secretary Dr Yunus Muftu P K 35 Yenisehir Ankara Authorized travel agent Wagons/Lits Cook Ziya Gokalp 20/C Ankara

ASSOCIATION FOR PAEDIATRIC EDUCATION IN EUROPE

The 1973 annual meeting of the Association for Paediatric Education in Europe will take place in Prague on September 9 and 10 For information please write to the Secretary General Professor Spyros Doxiadis Institute of Child Health Athens 608 Greece

Acta Pædiat Scand 62 333–336 1973

URINARY PHENYLALANINE EXCRETION IN HYPERPHENYL ALANINEMIC CHILDREN

F GÜTTLER and F ROSLEFF

From the John F Kennedy Institute Glostrup and the Institute for Mental Defectives for South East Jutland Brejning Denmark

The pathogenesis underlying the high phenyl alanine tolerance in persistent hyperphenyl alaninemia is still unknown Lines & Waisman (3) studied the twentyfour hour excretion of amino acids in phenylketonuric patients on and off diets and in hyperphenylalaninemic individuals Compared with normal children the phenylketonuric and hyperphenylalaninemic patients had aminoaciduria, whether on or off diet Lines & Waisman (3) suggested that hyperphenylalaninemic patients are phenyl ketonuric individuals who are protected by their increased urinary excretion of phenyl alanine

In order to examine this hypothesis further we have performed 24-hour phenylalanine loading tests on 5 children with persistent hyperphenylalaninemia The urinary excretion of phenylalanine after loading of these chil dren was compared with the excretion after loading of phenylketonuric children on diet parents of these children and normal individ uals

MATERIAL AND METHODS

The diagnosis of persistent hyperphenylalaninemia was proposed in children having hyperphenylalaninemia (fasting levels above 150 μmol/l) no phenylketones in the urine and after loading with phenylalanine no rise in serum tyrosine The diagnosis was confirmed by the extended phenylalanine loading test previously described (cf Güttler & Wamberg (2)) i e serum phenylalanine concentrations returning to preloading levels within 24 hours after a loading test dose of 0 1 g L-phenylalanine per kg of body weight (2) The diagnosis of phenylketonuria was proposed in children having fasting levels of serum phenylalanine above 150 μmol/l and phenylpyruvic acid in the urine The diagnosis was confirmed by phenylalanine loading i e serum phenylalanine above 600 μmol/l within 24 hours after the loading

The ages of the 5 children with hyperphenyl alaninemia ranged from 5 months to 5 years and the ages of the 8 phenylketonuric children ranged from 6 months to 11 years The ages of the heterozygotes were 2 years to 47 years and of the normal individuals 2 years to 36 years The 8 phenylketonuric children on diet were given a low-phenylalanine diet with Albumaid® enriched with methionine leucine iso leucine and tyrosine as the main source of protein The diet was supplemented with vegetables from the fourth month of life and with low phenylalanine bread from the eighth month of life The daily intake of phenyl alanine was restricted in order to keep fasting serum phenylalanine concentrations within 180–360 μmol/l

L-phenylalanine for loading was completely dis solved in 0 01 N HCl and the pure solution was given orally in a dose of 0 1 g per kg body weight The first meal was given 5 hours after the load test dose Blood samples were drawn at 1 2 3 4 and 24 hours after the loading The morning urine was collected before the load test and from then on the urine was collected separately for intervals of 0–6 hours and 6–24 hours after loading

Serum phenylalanine was determined by an adapta tion (2) of the fluorometric method of McCaman & Robins (4) using 25 μl of serum Serum tyrosine was determined by a microadaptation of the fluorimetric method of Udenfriend (9) using 150 μl of serum (2) The Aminco SPF 125 spectrofluorometer was used Creatinine was determined according to Popper's method (6) using the Boehringer modification Urin ary phenylalanine and tyrosine was determined on a

Part of this material was presented at The Danish Paediatric Society Meeting May 1971 (see Acta Paediat Scand 61 383 384 1972)

Table 1 *Serum concentration of phenylalanine and tyrosine before and during phenylalanine loading*

n	Hours after loading	Serum Phenylalanine[a] (μmol/l)	Tyrosine[a] (μmol/l)
Normals			
8	0	115 (80–140)	62 (51–92)
	1	595 (450–930)	141 (89–212)
	24	105 (65–135)	69 (54–114)
Heterozygotes			
12	0	120 (95–165)	64 (36–81)
	1	750 (595–1 010)	83 (46–148)
	24	120 (80–165)	60 (34–88)
Persistent hyperphenyl-alaninemia			
5	0	300 (115–405)	56 (47–68)
	1	825[b] (600–1 600)	55 (47–81)
	24	285 (110–360)	50 (41–120)
Phenylketonurics			
8	0	300 (145–560)	56 (39–119)
	1	1 005[b] (835–1 800)	54 (28–94)
	24	785 (610–1 650)	48 (31–70)

[a] Median and range in parentheses given below
[b] $p > 0.1$ (The Mann-Whitney U test (8))

Technicon automatic amino acid analyzer employing 140 × 0.6 cm glass columns packed with the Technicon Type B (17 μ) resin Lithium citrate buffers were prepared as described (5) The column was operated at 35 C for the first 6 / hours and at 70 C for the rest of the chromatogram

RESULTS

When completely dissolved, L phenylalanine was given orally to normal individuals or parents of phenylketonuric children the load of 0 1 g phenylalanine per kg of body weight was associated with an increase in serum tyrosine (Table 1) The serum concentration and urinary excretion of tyrosine was lower in heterozygotes than in normal individuals (Table 1 and 2)

The response of 5 children with persistent hyperphenylalaninemia to the phenylalanine load appeared as far as tyrosine is concerned to be the same as in phenylketonurics, i e no increase in serum tyrosine (Table 1) Although this seems to indicate a lack of phenylalanine hydroxylation to tyrosine, the serum phenylalanine concentrations returned to preloading levels within 24 hours (Table 1) In contrast, phenylketonurics take about 14 days to eliminate this load (cf Guttler & Wamberg (2))

During the first 6 hours after phenylalanine loading children with persistent hyperphenylalaninemia excreted significantly less phenylalanine than did children with phenylketonuria ($p < 0.01$ Table 2) In the interval between 6

Table 2 *Urinary excretion of phenylalanine and tyrosine before and during phenylalanine loading*

n	Hours after loading	Urine Phenylalanine[a] (μmol/g creat)	Tyrosine[a] (μmol/g creat)
Normals			
8	0	33 (23–370)	51 (24–406)
	6	222 (171–380)	159 (60–230)
	24	61 (36–113)	117 (43–266)
Heterozygotes			
12	0	43 (25–111)	35 (17–89)
	6	312 (132–653)	67 (37–204)
	24	93 (57–196)	53 (33–107)
Persistent hyperphenyl-alaninemia			
5	0	390 (280–1 014)	145 (38–290)
	6	929[b] (185–1 077)	96 (18–302)
	24	857 (508–922)	100 (68–187)
Phenylketonurics			
8	0	285 (69–1 151)	192 (33–272)
	6	1 314[b] (1 047–2 951)	171 (73–264)
	24	1 538 (433–3 686)	115 (54–390)

[a] Median and range in parentheses given below
[b] $p < 0.01$ (8)

hours and 24 hours after phenylalanine loading children with persistent hyperphenylalaninemia excreted approximately half the amount of phenylalanine excreted by phenylketonurics (Table 2) The high values of urinary tyrosine observed in phenylketonurics at time zero may be due to the high content of tyrosine in the diet In contrast to the normal individuals and heterozygotes loaded with phenylalanine tyrosine excretion decreased in phenylketonurics during the first 24 hours after loading (Table 2) The high zero values of urinary tyrosine in hyperphenylalaninemic children is so far unexplained (Table 2)

DISCUSSION

The present data do not support the suggestion forwarded by Lines & Waisman (3) that hyperphenylalaninemic patients are protected by their increased urinary excretion of phenylalanine The excretion of phenylalanine during phenylalanine loading of children with persistent hyperphenylalaninemia was less than that of phenylalanine loaded phenylketonuric children The tubular reabsorption of phenylalanine has a high capacity (1 7) and phenylalanine seems to be reabsorbed even more completely in phenylketonuric individuals than in normals (1) Three alternative mechanisms may be responsible for the high phenylalanine tolerance of children with persistent hyperphenylalaninemia they may metabolize phenylalanine by a route alternative to *p*-hydroxylation to tyrosine e g *o* hydroxylation they may eliminate phenylalanine by excessive urinary excretion or they may be able to form small amounts of tyrosine The first mechanism *o*-hydroxylation was excluded in a previous investigation (2) The second mechanism excessive urinary excretion of phenylalanine is not supported by the present investigation

SUMMARY

24-hour phenylalanine loading tests were done in 5 children with persistent hyperphenylalaninemia off diet The urinary excretion of phenylalanine were compared to the excretion after loading of phenylketonuric children on diet

Serum phenylalanine of children with persistent hyperphenylalaninemia returned to preloading levels (285 μmol/l) within 24 hours These children however excreted phenylalanine during phenylalanine loading in lesser quantities than patients with phenylketonuria on diet Serum phenylalanine of phenylketonuric children on diet attained preloading levels (300 μmol/l) approximately 14 days after loading Thus the present data do not support the hypothesis that hyperphenylalaninemic patients are phenylketonurics protected by increased urinary excretion of phenylalanine

ACKNOWLEDGEMENT

This study was supported by grants from the Research Committee of the Danish Mental Retardation Service (Project no 93)

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When completely dissolved L phenylalanine was given orally to normal individuals or parents of phenylketonuric children the load of 0.1 g phenylalanine per kg of body weight was associated with an increase in serum tyrosine (Table 1) The serum concentration and urinary excretion of tyrosine was lower in heterozygotes than in normal individuals (Table 1 and 2)

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Phenylketonurics			
8	0	285 (69–1151)	192 (33–272)
	6	1314[b] (1047–2951)	171 (73–264)
	24	1538 (433–3686)	115 (54–390)

Median and range in parentheses given below
[b] $p < 0.01$ (8)

Acta Pædiat Scand 62 337–340 1973

JUVENILE POLYPS OF THE RECTUM AND COLON

H TOCCALINO E GUASTAVINO F DE PINNI J C O'DONNELL
and M WILLIAMS

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Buenos Aires Argentina*

Rectal bleeding in children can be quite alarming for the family as well as for the physician B tween the ages of 2 and 6 years rectal bleeding without pain is a fairly frequent symptom usually associated with juvenile polyps Nevertheless this condition easily escapes early diagnostic considerations probably due to the lack of emphasis given to it

The purpose of this paper is to elucidate the incidence of this disease and to analyse the principal clinic signs facilitating early diagnosis and selection of treatment method

MATERIAL AND METHODS

Fifty cases were studied of a total of 4 000 gastroenterological consultations (an incidence of 1/80) over a p riod of 6 years The age range was 21 months to 11 years with a peak between 3 and 4 years 28 ere males and 22 females (Fig 1)

Rectosigmoidoscopy was performed on all patients Those who showed diffi ulties in collaborating with this procedure were given sodium Seconal Conventional rectoscopes with proximal illumination were used On 10 patients a colon contrast X ray with evacuation and inflation was carried out previous to th endoscopy

Serial parasitolog al stool examinations were made on 1 of these patients

The treatment consisted of polypectomy (via endoscopy) via the *Espeche* method (3) or fulguration or through a combination of these procedures Th Espeche method employs a metallic tube (40 cm x 3 mm) through which is passed a multifilament wire to form a cold loop distally with the 2 free ends proximally The wire used is Stainless Multistrand Surgical Sutures Size 0 (David and *Geck* Division American Cyanamid Company Danbury Conn)

All polyps were studied macroscopically

RESULTS

All patients showed slight symptomatology of which rectal bleeding was a constant finding (Fig 2) Abdominal pain was present in 7 patients and abdominal pain plus diarrhea in 3 a polyp-prolapse passed the anus in 4 patients Rectal bleeding following normal bowel movements was thus the only sign in the rest of the material (35 patients) One patient had however a polyp prolapse without rectal bleeding

The polyps were localized within the last 20 cm of the colon with preference for the last 5 cm (Fig 3) One patient presented 2 polyps and another 3 in both patients all polyps were on single pedicles The remaining 48 patients had single polyps 2 of these may however be con idered as recurrences since the patients had been polypectomized 2 years earlier Polyp size ranged from 3 to 20 mm in diameter The polyps were round in shape (Fig 4) wine red in colour and they were rather hard with easily ruptured blood vessels Histologically they were harmartomas (Figs 5 and 6) 15 of them showed signs of infection

Of the 12 cases studied parasitologically 6 were negative 3 had oxyuris vermicularis 2 trichuris trichiura and 1 giardias

DISCUSSION

The age incidence curve of the present material shows a maximal frequency between 2–6

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9 Udenfriend S *Fluorescence assay in biology and medicine* Academic Press London and New York 1966 p 130

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Accepted Nov 23 1972

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Gl Landevej 7
DK 2600 Glostrup
Denmark

Key words Phenylalanine tolerance in persistent hyperphenylalaninemia phenylalanine and tyrosine excretion extended phenylalanine loading



Fig. 4. The polyps of patient A. C. (age 3 years).

ment. The constancy of this sign indicates that juvenile polyps should be suspected in all cases of rectal hemorrhage. The complete history and a physical examination, including rectal palpation, may often yield the presumptive diagnosis.

A prolapsed polyp may sometimes be difficult to distinguish from a rectal prolapse; however, the latter occurs more frequently in very young children in whom polyps are rare (Fig. 1).

All polyps were observed to have pedicles and a common histological appearance (hamartomas) and they generally occurred singularly (10).

Spontaneous disappearance may occur as a result of torsion followed by vascular changes and a falling off of the tumor. Such a procedure is probably related to tumor size and pedicle length.

The ethiology is still under discussion. Opinions coincide, however, as to embryological character of the formation, quite different from that of the adenomas seen in later life (6, 11). The congenital aspect is supported by the greater incidence in the first years of life and by the fact that they are only exceptionally seen after the age of 12 years.

Both techniques used in this study for extirpation of the polyps have advantages and disadvantages. The Espeche method has as yet not produced any difficulties in our service, but it carries, of course, the risk of profuse bleeding if the pedicle is cut off. Subsequent fulguration may be difficult. The procedure performed with multifilament wire loop partially solves this problem, since it aids hemostasis. The Espeche method is the technique selected for large polyps extirpated in a single intervention. By the Espeche method adequate material is always obtained for histology. Fulguration of the pedicle (if observable) or—at various sessions—of the tumor body, obviously cause inconvenience to the patient, and furthermore, by this technique it is often difficult to obtain adequate material for histology.

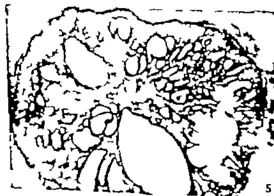


Fig. 5. Microscopic picture (H. & E. ×1.5) of the polyps of patient J. L. (age 4 years). Note the dilated glands and the cystic spaces.



Fig. 6. Microscopic picture (H. & E. ×100) of the polyps of the patient J. L. (age 4 years). Note the dilated glands and the infiltration of lymphocytes and polymorphonuclear leucocytes of the stroma.

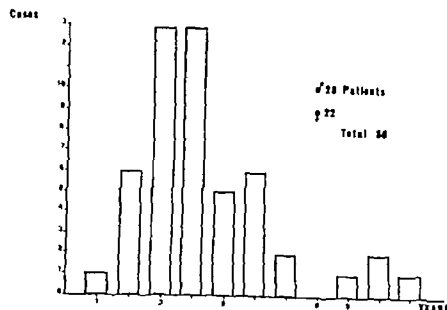


Fig 1 Age distribution of 50 cases of juvenile polyps

years with a peak between 3–4 in agreement with most other reports (1 4 5 7 8 9 12 13 15 17) The pediatric literature in general gives this condition a frequency lower than that observed by us The explanation for this may be spontaneous disappearance of the polyps (see below) the lack of routine endoscopic examination in childhood or that the habitu radiological techniques usually do not reve the tumor (2 14 16) Of the 10 cases examin roentgenologically in the present material o one revealed the presence of the tumor T authors feel therefore that endoscopy is better method than X ray furthermore, th latter procedure is not innocuous and wh positive it does not offer more informatio than endoscopy Barium enema of course h its place in the diagnosis of polyps above th area available for endoscopic examination

Of the 50 cases studied 49 showed non pai ful rectal bleeding appearing as drops im mediately following a normal bowel move

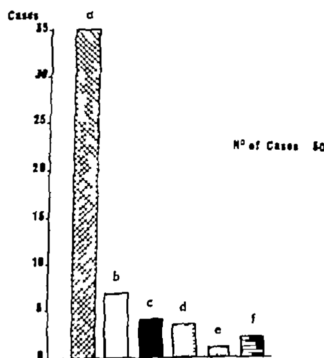


Fig 2 Symptomatology of 50 cases of juvenile polyps *a* rectal bleeding *b* rectal bleeding + abdominal pain *c* rectal bleeding + polyp prolapse past the anus *d* rectal bleeding + mucous diarrhea *e* polyp prolapse *f* rectal bleeding + spontaneous polyp elimination

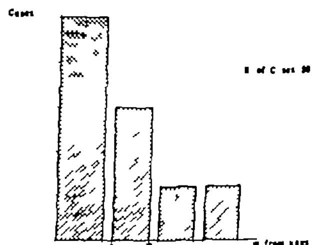


Fig 3 Localization of the polyps

Acta Pædiat Scand 62 341–348 1973

PAROXYSMAL TACHYCARDIA IN INFANCY AND CHILDHOOD

I *Paroxysmal Supraventricular Tachycardia*

ELLEN DAMGARD ANDERSEN J RAMSØE JACOBSEN E SANDØE, J VIDEBÆK and A WENNEVOLD

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Only a few larger series of infants and children with supraventricular tachycardia (SVT) followed for a reasonable period of time have been published So far the largest series have been presented by Nadas & co workers (7) Lundberg (3) Keith (1) and lately by Simcha & Bonham-Carter (11) who contributed 38 54 74 and 39 cases respectively

We have collected a series of 62 infants and children with SVT followed for periods ranging from one to 30 years Fifty four of them had experienced attacks of SVT of less than 1 month s duration and these are the subject of this paper on *paroxysmal* SVT The remaining 8 patients have been classified as cases of *permanent* or *repetitive* SVT as their attacks lasted for longer periods of time i e from 7 months to 8 years They will be described in a later publication

MATERIAL AND METHODS

The series has been collected from two departments of cardiology and three departments of paediatrics all of which belong to university hospitals Case records and lists of diagnoses of two to three decades have been scrutinized and all patients with paroxysms of SVT of less than 1 month s duration and with onset before the age of 15 years have been included in the study

Episodes of tachycardia have been documented by ECG recordings in 50 of the 54 cases These ECGs showed the following characteristics

(*a*) typical SVT in 45 cases, i.e. normal QRS complexes together with distinct ectopic atrial activity and/or a heart rate above 200/min

(*b*) in 5 cases broad QRS complexes of another configuration than that recorded during normal sinus rhythm i e changes which might be due to either SVT with aberration block or ventricular tachycardia However we have considered it justified to classify the tachycardia as supraventricular as a close look at all available ECG recordings did not reveal any further evidence favouring the diagnosis of ventricular tachycardia, i e atrioventricular dissociation with faster ventricular than atrial rate and/or ventricular or atrial captures or fusion beats

The remaining 4 children without ECG-documented episodes of tachycardia had the Wolff Parkinson White anomaly (WPW) They all had a prolonged history of attacks of rapid palpitation which started after infancy at ages ranging from 4 to 13 years They have been included in this series as ventricular tachycardia is known to occur extremely rarely—if ever—in patients with WPW (6)

The diagnosis of WPW was based on the presence of short PR intervals broad QRS complexes and distinct delta waves occurring during normal sinus rhythm Normal values of PR and QRS for different age groups indicated by Ziegler (14) have been used in the evaluation of the ECGs

All 54 patients could be traced Follow up time ranged from 1 to 30 years None had died 52 came for a follow up examination which included ECG and chest roentgenogram while 2 patients answered a questionnaire

RESULTS

Relative incidence and age and sex distribution

During the preliminary phases of the study we found a total of 73 infants or children with

SUMMARY

Fifty rectal polyps were observed in 4 000 pediatric gastroenterological consultations (an incidence of 1/80) The age range was 21 months to 11 years with a maximal frequency from 2–6 years No sex difference was seen Rectal bleeding was the motive for medical consultation The diagnosis was made by recto-sigmoidoscopy The usual colon X ray techniques were not considered useful for diagnosis of juvenile polyps

A single polyp was observed in 46 patients, in the majority localized within 10 cm of the anus *Histology showed the characteristics of* hamartoma 15 of them also presented signs of infection

The treatment consisted of polypectomy through the Espeche method or fulguration or through a combination of both procedures

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Table 3. *Incidence of organic heart disease*

Sex	Type of heart disease	Age at: Onset of attacks	Latest attack	Follow up	WPW
♂	Atrial septal defect	A few days	1 month	5 years	−
♀	Cardiomyopathy	3 months	3 months	5 years	+
♂	Fallot's tetralogy	4 months	8 years	8 years	−
♀	Coarctation of aorta	6 months	26 years	26 years	+
♂	Cardiomyopathy	8 months	4 years	7 years	+
♂	Ebstein's anomaly	<1 year	26 years	26 years	+
♂	Acute myocarditis suspected	3 1/2 years	4 years	29 years	−
♀	Corrected transposition	7 years	21 years	23 years	+
♀	Ebstein's anomaly	13 years	22 years	22 years	+

cardia could be identified. In the remaining 4 patients the mechanism was atrial flutter or fibrillation (Fig. 2).

In the majority of the patients—also in most of the patients with WPW—the ECG during tachycardia showed normal QRS complexes (cf. Fig. 2), while broadened QRS complexes due to aberration block have been observed in 5 cases only, 3 with and 2 without WPW (Fig. 3).

Symptoms and signs during tachycardia

In the infants the most common symptom noted before admission was a change of colour. Thus cyanosis or pallor had been seen in 24 of the 28 infants. Other symptoms reported were rapid peripheral or precordial pulsation, rapid respiration, vomiting, refusal to eat and weakness. Each of these symptoms had occurred in about half of the infants, most of whom had had two, three or more symptoms.

In only 7 infants was paroxysmal tachycardia indicated as diagnosis of admission by the referring physician. Other common diagnoses were cardiac disease (5 cases), attacks of cyanosis (6 cases) and gastrointestinal disease (5 cases).

The physical sign most frequently noted on admission in hospital was hepatomegaly which gradually subsided after the attack of tachycardia. This was found in 19 infants. Five of

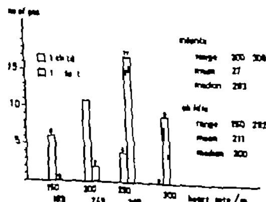


Fig. 1. Maximum heart rates during tachycardia in 49 patients—28 infants and 21 children. In one patient—not included in the figure—tachycardia has been seen only on an oscilloscope.

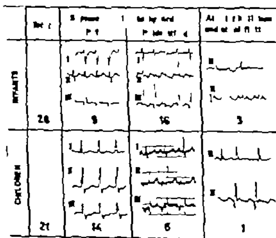


Fig. Incidence and different types of supraventricular tachycardia in the infants and the children.

Table 1 *Relative incidence of supraventricular (SVT) and ventricular tachycardia (VT) in infants and children*

	SVT		
	Paroxysmal	Repetitive persistent	VT
Infants	28	2	1
Children	26	6	10
Total	54	8	11

tachycardia 62 had SVT (paroxysmal in 54 and repetitive or persistent in eight) and 11 ventricular tachycardia (Table 1)

Twenty eight of the 54 patients with *paroxysmal* SVT had their first attack in infancy and in two thirds of these the attacks started within the first month of life There was a male predominance in the infant group whereas the majority of the children were female (Table 2) However the differences were not statistically significant

Predisposing and precipitating factors

Birth weight below 2 500 g was found in three patients i e 5% which does not differ from the expected frequency

Organic heart disease was observed in 9 cases (Table 3) Six patients had congenital cardiac anomalies 2 infants had cardiomyopathy (persistent cardiomegaly and ECG abnormalities for years congenital malformations excluded by catheterization) and in one child acute myocarditis was suspected Signs of heart failure cardiomegaly or ECG

Table 2 *Age at onset of paroxysmal SVT*

	♂	♀	Total
0–30 days	12	6	18
1–4 months	1	3	4
4–12 months	3	1	4
Unknown but <1 year	2	0	2
No of infants	18	10	28
1–6 years	1	6	7
6–14 years	8	11	19
No of children	9	17	26
Total no of patients	27	27	54

changes was not observed in the latter patient but 5 months before his attacks of tachycardia started he had had measles after which he felt weak and tired for months He had several attacks of tachycardia at a rate of 280–300/min during a 6 month period since when he has been well and fit

At follow up 30 of the 54 patients reported that their attacks could be provoked by one or more of the following factors Twelve patients mentioned exertion 3 excitement or nervousness 8 a variety of other factors such as fever some special movement, hiccups and high and low environmental temperature and 7 mentioned two or more of these factors as being able to precipitate attacks

ECG abnormalities between attacks of tachycardia

A WPW pattern has been observed in a total of 30 patients (56 %) 14 infants and 16 children respectively Fourteen had WPW in all ECGs recorded 16 had the anomaly intermittently At follow up 1 to 30 years after onset of tachycardia WPW was present in 20 cases while 8 patients had a normal excitation pattern Two of the patients have no ECG follow up WPW coincided with congenital heart disease in 4 cases only

The Lown Ganong Levine syndrome (2) was diagnosed in 1 patient Two patients with congenital heart disease had right bundle branch block alternating with WPW One patient with a normal heart had incomplete right bundle branch block Four patients had a left posterior hemiblock pattern which alternated with WPW in one (13)

ECG during tachycardia

On the average the rates in infants exceeded that of children by 64 beats/min (Fig 1) There was no significant difference of heart rates in patients with and without WPW

In 23 patients no definite P waves could be detected in the ECGs recorded during tachycardia In another 22 patients P waves indicating atrial

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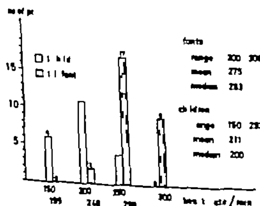


Fig 1 Maximum heart rates during tachycardia in 49 patients— 28 infants and 21 children In one patient—not included in the figure—tachycardia has been seen only on an oscilloscope

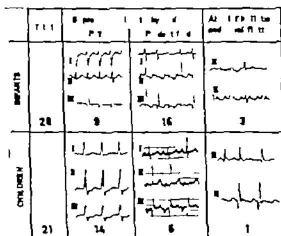


Fig 2 Incidence and different types of supraventricular tachycardia in the infants and the children

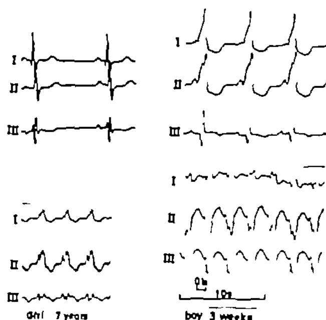


Fig 3 Aberration block during paroxysms of tachycardia Left ECG of 7 year old girl during normal sinus rhythm (*upper*) and during supraventricular tachycardia (*lower*) with aberrant intraventricular conduction resulting in broad QRS complexes Right ECG of 3 week old boy with WPW during normal sinus rhythm (*upper*) The QRS complexes during tachycardia (*lower*) are widened and quite different from WPW complexes of sinus rhythm

these also had oedema and another 8 also had cardiomegaly and/or signs of pulmonary congestion One infant had cardiomegaly but no hepatomegaly 6 had only cyanosis and/or tachypnoea and 1 infant had signs attributable to heart failure

Among the *children* the most commonly recorded symptom was palpitation which occurred in 25 of the 26 children Accordingly the diagnosis on admission was paroxysmal tachycardia in all cases Thirteen children complained of tiredness 10 of dyspnoea and 7 felt dizzy during attacks Precordial sensations were reported in 7 nausea or vomiting in 3 and abdominal pain in 2 patients Four children had had one or more short lasting syncopes during attacks None of the children showed signs of congestive heart failure

Treatment of attacks

Although most of the *infants* were very seriously ill with congestive heart failure on admission considerable improvement followed the initiation of digitalis treatment which was given to 25 infants shortly after ECG verification of tachycardia irrespective of the presence or absence of WPW In most cases digitalis was given alone in a few together with various antiarrhythmic drugs (e g neostigmine and quinidine) In nearly all cases sinus rhythm was restored within hours or a day Two thirds of the infants had repeated episodes of tachycardia during the following days weeks or months, but signs of congestion rarely reappeared as long as digitalization was maintained

In the *children* treatment of attacks included reflex vagal stimulation digitalization and various antiarrhythmic drugs Many children were not started on any drug treatment during attacks Sinus rhythm usually returned within hours, but only in a minority of cases could thus be attributed to treatment

At follow up 15 patients, of whom only two were still children described various physical manoeuvres such as the Valsalva manoeuvre deep breathing breath holding and a sort of squatting enabling them to abolish paroxysms with varying success

Prevention of attacks

Digitalis has been used to prevent attacks in a total of 38 patients while 10 received quinidine Other antiarrhythmic drugs have been used in a few patients The exact frequency of attacks during and after treatment cannot be evaluated from our data but it can be stated that recurrences occurred whether or not any preventive treatment was given

Prognosis

All the 54 patients were alive when the follow up study was performed

The *infant group* of 28 patients was followed up after 1 to 26 years Fifteen (54%) had no attacks after infancy Of 23 patients who were followed for at least 5 years (mean 11 and median 9 years) 17 (74%) had had no attacks during the 3 years preceding the fol

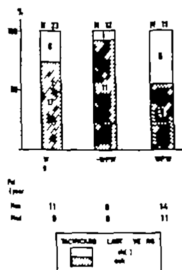


Fig 4 Recurrence rate of paroxysmal tachycardia in 23 infants followed for at least 5 years Inserted figures indicate absolute numbers

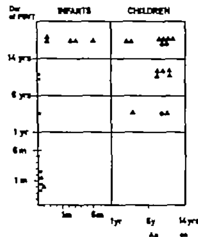


Fig 5 Duration of period with attacks of tachycardia in relation to age at onset for 23 infants and 26 children who have been followed for at least 5 years ● no WPW no attacks during the last 3 years ○ no WPW still attacks of tachycardia ▲ WPW no attacks during the last 3 years. △ WPW still attacks exact onset in infancy unknown

low up examination (Fig 4) The tendency to recurrence was greater among patients with WPW Thus only 6 out of 11 patients had been free from attacks during the last 3 years as opposed to 11 out of 12 patients without this anomaly

From Fig 5 it can be seen that 13 of the 23 infants who were followed for at least 5 years had had their first attack during the first 4 months of life and their latest attack before the age of 6 months This was also true for another 2 patients who have been followed for 1 and 3 years respectively On the other hand early onset of paroxysmal tachycardia did not invariably mean early disappearance of attacks as one third of the infants who had their first attack before the age of 4 months continued to have attacks in some cases even until adult age (cf Fig 5)

Most of the 15 infants had a few recurrences on the average three and at most eight for periods of up to 4½ months (mean 1 month median 3 weeks) with early onset and disappearance of attacks

Four had only one single attack One of these had atrial flutter for 2 weeks and another had atrial fibrillation for 3 weeks

The group of 26 *children* comprised 23 patients who were followed for at least 5 years (mean 14 median 11 years) and of these only three had been free from attacks for at least 3 years at follow up (Fig 6) All the remaining 18 patients who were followed after childhood still had recurrences

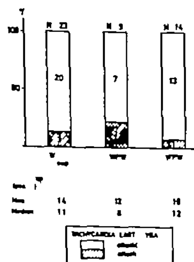


Fig 6 Recurrence rate of paroxysmal tachycardia in 26 children followed for at least 5 years Inserted figures indicate absolute numbers

DISCUSSION

The data presented do not allow any evaluation of the incidence of paroxysmal tachycardia among infants and children in the population The only estimate we have been able to find in the literature has been given by Keith et al (1) who stated that the incidence may be something like one out of 25 000 children As demonstrated in Table 1 the frequency of supraventricular tachycardia by far exceeds that of ventricular tachycardia in children and especially in infants

The male predominance reported by other authors (3 7 11) could not be substantiated by our data (Table 2) The incidence of congenital heart disease was 11% in our series compared with earlier published figures of 8 4 20 and 34% respectively (1 3 7 11) The percentage of patients with WPW in our series was as high as 56% while other authors have reported incidences ranging from 12–51% (1 3 7 11) Left posterior hemiblock which according to a recent publication may predispose to paroxysmal tachycardia (13) was observed in 4 cases

The heart rate during tachycardia was significantly higher in infants than in children a finding which may explain the severe cardiorespiratory distress usually observed in the former group

Furthermore it appears from our series that in infancy paroxysmal tachycardia may well be overlooked unless carefully searched for The patients histories are often inconclusive signs may at first glance seem unspecific and concomitant symptoms (e g from the gastrointestinal tract) may mislead the referring physician In view of the serious congestive heart failure so commonly seen in young infants, death may well ensue if the condition is misdiagnosed and appropriate treatment not instituted

Digitalis was the drug most commonly used to treat attacks of tachycardia in our series Sinus rhythm was restored within hours or a day but tachycardia often recurred The main importance of digitalis was undoubtedly its efficacy in the treatment of congestive heart failure during attacks of tachycardia in infants and its possible role in the prevention of congestive failure during recurrent attacks In our opinion this remains the main indication for digitalis in paroxysmal tachycardia in infancy and justifies the recommendation that digitalization should be continued for some months In older infants this may not be necessary and in children over the age of 1 year it is rarely needed Presence of WPW might according to some authors (10) have been considered a contra indication for the use of digitalis However no serious side effects of digitalis were observed in any of our cases

d c conversion had not been used in any of our patients all of whom improved satisfactorily on digitalis However in selected emergency cases d c shock would now be the treatment of choice (5)

Other authors agree on the value of digitalis in the treatment of heart failure but are reluctant to draw any conclusions concerning the value of preventive treatment except for single patients apparently controlled by some drug or a combination of drugs (4 7 11)

Although our data do not allow any detailed analysis of the frequency of attacks throughout the observation period it is our general impression that few—if any—patients did benefit from preventive therapy with digitalis or other antiarrhythmic drugs

There were no deaths in our series This corresponds well with Simcha s & Bonham Carter s findings (11) However other authors have found mortalities ranging from 2 to 11% most often due to other serious diseases including cardiac malformations (1 3 7) Nadas et al mention two deaths attributable to treatment of attacks (7)

Onset in infancy was most frequent in the first 4 months of life Two thirds of these babies had only a few attacks during the following weeks or months The remaining one third continued to get attacks after infancy and often after childhood Children usually

continued to get attacks until follow up the majority having been followed into adulthood These finding are in keeping with other authors (1 7 11) Patients with WPW had a somewhat greater tendency to recurrences than patients without this syndrome but the more favourable prognosis for infants compared with that of children was also found in patients with WPW

In conclusion it may be stated that paroxysmal supraventricular tachycardia is a rare condition in infancy and childhood which particularly affects young infants in whom it often produces heart failure which however can readily be controlled with digitalis Children rarely have severe symptoms Infants often outgrow the tendency to attacks but this rarely happens in children The results of preventive treatment are discouraging on the other hand after infancy the discomfort caused by the disease is usually limited A most important task for the physician should be to reassure the children and their parents that in the absence of organic heart disease the disorder is benign Exertion and excitement may in some cases provoke attacks but as a whole restriction of the patients physical activity is rarely needed and should be held at a minimum

SUMMARY

A 1 to 30 year follow up study of 54 infants and children with paroxysmal supraventricular tachycardia is presented In 28 cases the first attack occurred in infancy and in 18 of these already in the first months of life Nine patients had organic heart disease The WPW syndrome was diagnosed in 30 cases When first seen most of the infants presented signs of incipient or manifest congestive heart failure which was very unusual in the children most of whom had only minor symptoms Four children had experienced brief syncopes during attacks Digitalis was effective against congestive heart failure and when continued may have prevented failure during subsequent attacks Whether digitalis and other antiarrhythmic agents facilitated conversion to sinus rhythm could not be established in this study Vagal stimulation was only rarely effective Preventive treatment with digitalis or other antiarrhythmic drugs seemed to have little if any effect on the frequency of recurrent attacks Out of 23 infants who were followed for at least 5 years 17 had been free from attacks during the last 3 years and 13 of these had had their last attack before the age of 6 months Out of 23 children followed for 5 years or more only 3 had been free from recurrences during the last 3 years Patients with the WPW syndrome had a somewhat higher incidence of recurrent attacks

ACKNOWLEDGEMENT

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PAROXYSMAL TACHYCARDIA IN INFANCY AND CHILDHOOD

II *Paroxysmal Ventricular Tachycardia and Fibrillation*

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Paroxysmal ventricular tachycardia and fibrillation seem to be rare conditions in infancy and childhood To our knowledge 43 cases have been reported in the literature 39 of paroxysmal ventricular tachycardia (1 2 4–7 10–14 16 17 19 21–28 30 32 36) and 4 of fibrillation (3 8 18 20) Only nine of these case reports provide data pertinent to an evaluation of long term prognosis (1 11 14 16 27 30)

The present 3–20-year follow up study deals with 11 cases one infant and ten children with paroxysmal ventricular tachycardia or fibrillation It constitutes the second part of a follow up study of a total of 73 infants and children with paroxysmal tachycardia (9)

MATERIAL AND METHODS

This series of 11 patients with paroxysms of ventricular tachycardia (or ventricular fibrillation) with onset before the age of 15 years has been collected from two departments of cardiology and three departments of paediatrics (9) One patient (case no 9) had died after discharge but data from a visit in an out patient-clinic of cardiology half a year earlier allowed evaluation of her status prior to death The remaining 10 patients have all been traced and have come for a re-examination including interview physical examination electrocardiogram and a chest roentgenogram

The diagnosis of ventricular tachycardia has been affirmed by ECG recordings of episodes of tachycardia (comprising more than 10 beats) which all fulfilled the following criterion (*a*) abnormal broad QRS complexes which were different in shape from complexes observed during sinus rhythm

This criterion alone would not exclude cases of supraventricular tachycardia complicated with aberration block (31) and therefore it was considered mandatory that one—or preferably more—of the following criteria be fulfilled (*b*) atrioventricular dissociation with clearly defined independent atrial and ventricular complexes and an atrial rate less than the ventricular rate (*c*) ventricular captures and fusion beats (*d*) occurrence during sinus rhythm of single ventricular ectopic beats with the same configuration as the QRS complexes seen during tachycardia

RESULTS

Age and sex distribution

The age at onset the sex distribution and pertinent clinical data are seen in Table 1 Only 1 patient had his first attack in infancy There were 5 boys and 6 girls

Possible predisposing and precipitating factors

Additional heart disease was present in seven patients A diagnosis of idiopathic cardiomyopathy has been made in 3 children (cases 6 7 and 10) based on the presence of cardiomegaly—and in two cases also ECG abnormalities—persisting or progressing over a period of years A ventricular septal defect with a small arteriovenous shunt and normal pres-

3 – 73 874 *Acta Pæd Scand 62*

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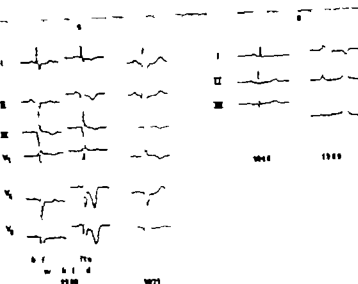


Fig 1 Abnormal ECGs between attacks of ventricular tachycardia in 2 patients with cardiomyopathy Paper speed 50 mm per sec 1 mV = 1 cm (in all figures)

precordial pain (2 cases) and postparoxysmal polyuria (2 cases) In the same group of patients the following physical signs were found cyanosis (3 cases) tachypnoea (3 cases) hepatomegaly (1 case) engorgement of the neck veins (1 case) and pulmonary oedema (1 case)

Sudden loss of consciousness with or without convulsions and incontinence of urine often but not always correlated to physical or emotional stress has been noted as the only symptom of tachycardia in three children (cases 3 5 and 6) Two of the children were initially treated for epilepsy and only the ECG monitoring during a fainting spell and the use of exercise ECG led to the diagnosis of Adams-Stokes attacks (34)

Finally one boy (case 2) experienced no symptoms and showed *no signs of* heart disease The tachycardia was diagnosed at a routine medical investigation when he was 2 months old and this boy now 12 years old has remained asymptomatic in spite of very prolonged attacks (the latest attack continued a fortnight at a rate of 140–180 per minute)

ECG abnormalities between attacks of tachycardia

The ECG was normal in 5 patients (Table 1) Three patients (nos 2 6 and 8) had incomplete right bundle branch block One of these (case 6) had in addition a deep Q_{III} and during work load RS-T depression and deep negative T waves (Fig 1) Another patient (no 9) developed RS T depression and negative T_I and deep Q_{III} (Fig 1) at rest The latter 2 patients were classified as having cardiomyopathy

Bigeminal rhythm has been observed during longer periods in 2 patients (cases 4 and 10) during and following acute myocarditis but subsided over a period of 2–3 years Ventricular ectopics occurred with increasing frequency on exercise in 5 patients (cases 3 4 5 6 and 10) Wandering pacemaker was recorded in 2 patients (nos 3 and 5) in one of them (no 5) there was a constant sinusbradycardia and also occasionally a slow nodal rhythm with a rate as low as 28 per minute

ECGs during tachycardia

Eight children had had attacks which usually lasted for 2–3 hours more seldom for days (Figs 2 and 3) The ventricular origin of the tachycardia was in all cases confirmed by the observation of atrioventricular dissociation (Fig 2) In 2 patients ventricular captures or fusion beats were also present (Figs 2 *B* and 3 *A*) and in 4 the similarity between the QRS complexes of tachycardia and of single ventricular ectopic beats during sinus rhythm

Table 1 *Pertinent clinical data from 11 patients with paroxysmal ventricular tachycardia or fibrillation*

Case no	Sex	Age at onset (years)	Additional heart disease	Type of tachycardia	ECG between attacks	Follow up time (years)
1	M	3	None	Repetitive	Normal	20
2	M	2/12	None	Prolonged	Incomplete RBBB	12
3	F	6	None	Brief malignant	Normal	9
4	F	12	Myocarditis	Repetitive	Bigeminy	3
5	F	8	Myocarditis (measles)	Brief malignant	Bradycardia, nodal rhythm	11
6	M	7	Cardiomyopathy VSD	Brief malignant	Incomplete RBBB Q_{III} ST_{III} depres	6
7	F	1	Cardiomyopathy	Repetitive	Normal	5
8	M	1	None	Repetitive	Incomplete RBBB	17
9	F	12	Cardiomyopathy	Repetitive	Negative T_I ST depres	6
10	F	6	Rheumatic myocarditis	Repetitive	Normal	9
11	F	11	VSD	Repetitive	Normal	6

VSD = ventricular septal defect
RBBB = right bundle branch block

sures in the right side of the heart was present as an additional feature in one (case 6) Three children most likely had had myocarditis at the start—or just prior to the start—of paroxysmal tachycardia In two of them (cases 4 and 10) the debut of tachycardia coincided with a period of fever signs of congestion and cardiomegaly and in one case also with rheumatic activity The symptoms developed quite suddenly and subsided over a period of 1 and 3 months respectively The third case of suspected myocarditis (case 5) occurred in a girl who at the age of 8 years developed measles associated with high fever and cerebral symptoms of some days duration Three months later she had her first syncopal attack (most likely due to an episode of ventricular fibrillation) ECG—now recorded for the first time—showed bradycardia, wandering pacemaker and periodically nodal rhythm Finally one child (no 11) had a ventricular septal defect with a medium sized arteriovenous shunt

$$\left(\frac{\text{pulmonary flow}}{\text{systemic flow}} = 2\ 0\right)$$

normal pulmonary pressure and normal end diastolic pressures in both ventricles

In four patients (cases 3 5 6 10) paroxysms could be provoked by exertion and in 3 patients (cases no 3 5 10) emotional disturbances were mentioned as precipitating factors In 4 patients (cases no 5 7 10 11) an accumulation of attacks could be seen during minor febrile diseases Precipitating factors could thus be found in 6 of the 11 patients

Symptoms and signs between attacks

Cardiac symptoms and signs had been present between attacks due to the described additional heart disease in 7 children In the remaining 4 children without additional heart disease functional capacity was significantly limited in the two a limitation most likely caused by the experience that exertion might provoke attack of tachycardia

Symptoms and signs during tachycardia

In 7 patients the following symptoms were encountered at attack palpitation (5 cases) tiredness and weakness (4 cases) nausea and abdominal pain (3 cases) dyspnoea (2 cases)

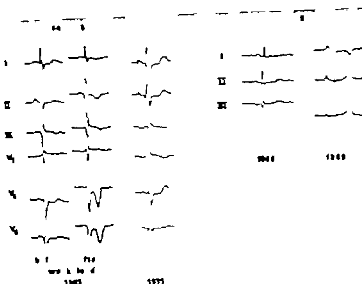


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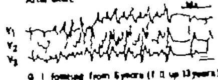
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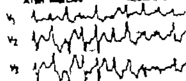










Fig 4 Top trace ECG immediately after heavy exercise (case 3) showing runs of multifocal ventricular extrasystoles with a few normal beats at the beginning and at the end Middle trace ECG immediately after exercise (case 5) showing runs of multifocal ventricular extrasystoles Lower tracing ECG monitoring with telemetry (case 6) before and during syncopal attack The strips are not continuous Sinus tachycardia is followed by ST depression and ventricular fibrillation

children (*cases 3 5 and 6*) who suffered from recurrent fainting spells As indicated by the upper two tracings in the figure heavy work on a bicycle *ergometer* resulted in short episodes of ventricular tachycardia or bursts of multifocal ventricular ectopics The stress provoked tachycardia of these patients usually changed to normal sinus rhythm in a few minutes or less Furthermore continuous ECG monitoring in the third child (lower part of Fig 4) demonstrated an occasional conversion to ventricular fibrillation resulting in syncope

Table 2 *Associated heart disease and features of paroxysmal ventricular tachycardia or fibrillation*

		Features		
		Prolonged		
	Total	Repetitive	Continuous	Brief malignant"
Idiopathic cardiomyopathy	3	2	—	1
Myocarditis	3	2	—	1
Congenital heart disease	1	1	—	—
No heart disease	4	2	1	1
Total no	11	7	1	3

for which reason the tachycardia has been classified as brief malignant tachycardia

No correlation could be demonstrated between type of additional heart disease and type of ventricular tachycardia (Table 2)

Prevention of attacks

Prophylactic drug treatment has been given to 10 of the 11 patients The three patients with syncopes and brief malignant tachycardia were treated with propranolol and a reduction in the frequency of the attacks was obtained (34) Six patients were treated with quinidine or procainamide and/or digoxin the treatment was at times followed by a decrease in the frequency of attacks However complete freedom from attacks was *not achieved* and in one patient (case 10) a definite increase was observed

A few patients seemed to benefit from treatment with digoxin (cases 4 9 and 11) However at the time of treatment all of them had some degree of congestive heart failure

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↑ p wave   S sinus beat   F fusion beat   SA sinoatrial block

Fig 2 Examples of ventricular tachycardia Trace A (case 11) shows abnormal QRS complexes and A V dissociation Trace B (case 7) shows in addition a fusion beat and the trace starts and ends with a sinus beat

provided further support of the diagnosis (Fig 3 *B*) Ventricular tachycardia continuing for hours without any intervening periods of sinus beats has been observed in 1 patient only In this case (case 2) atrioventricular dis sociation was not present as the ECG showed retrograde P waves following the broadened QRS complexes However the finding in one ECG tracing of ventricular captures and fu sion beats was taken to indicate a ventricular origin of the tachycardia

Fig 4 shows ECGs from the remaining 3

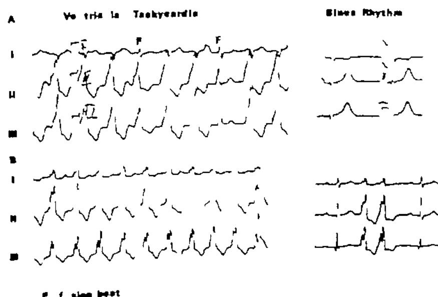


Fig 3 Examples of ventricular tachycardia Trace A (case 9) shows two types of fusion beats The normal sinus beat is shown to the right Trace B (case 10) shows the similarity between the QRS complexes during tachy cardia (*left*) and the QRS con figuration of ventricular ectopic beats (*right*)

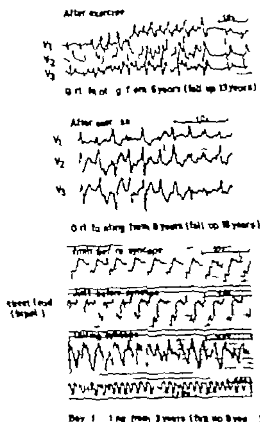


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A

I

II

↑ ↑ ↑ SA ↑ ↑ ↑

III

B

S F S

I

II

III

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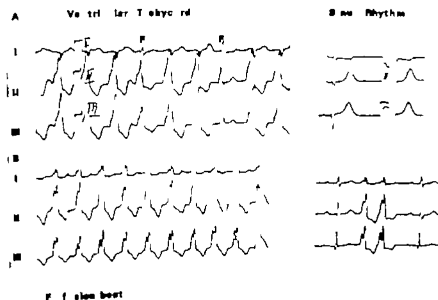
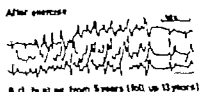


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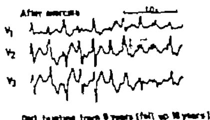




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Myocarditis	3	2	—	1
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Table 3 *Duration of paroxysmal ventricular tachycardia (PVT)*

Associated heart disease	Duration of single attacks Hours			Duration of period with PVT Years			Frequency of attacks during illness Mean number of attacks per year (clinically pertinent) Attacks/year		
	<1	1–24	>24	<1	1–2	>3	<2	2–10	>10
Idiopathic cardiomyopathy	1	2		1		2		2	1
Myocarditis	2	1		1	1	1		2	1
Congenital heart disease		1				1			1
No heart disease	2	1	1	2		2	2	1	1
Total	5	5	1	5		6	2	5	4

d c defibrillation had been used only once (case 6) with termination of ventricular fibrillation as the immediate result

In 5 patients several attacks of ventricular tachycardia terminated spontaneously

Prognosis

The patients have been followed up after 3 to 20 years with a mean of 9½ years (Table 1)

One patient with cardiomyopathy (case 9) had died at the age of 18 years, 6 years after the initial cardiac symptoms During her disease there had been progressive ECG changes (Fig 1) and persistent cardiomegaly On quinidine treatment she had one or two attacks of tachycardia per year She died suddenly during an attack At autopsy the myocardium was hypertrophied with diffuse fibrosis

All 3 patients with brief malignant tachycardia have had potentially dangerous syncopal attacks followed by sustained unconsciousness They have so far survived but one (case 6) has had to be resuscitated with d c defibrillation

The attacks of the remaining 8 patients were at no time considered to be life threatening

The duration of the single attacks of tachycardia the duration of the period with tachycardia and the mean frequency of attacks during illness are shown in Table 3 One patient had attacks of considerable length (2 to 3 weeks) in the remaining patients the mean duration of attacks was 2–3 hours

The period with attacks of tachycardia have ceased in 5 of the patients within 2 years after onset In 1 patient however 9 years elapsed between the first and the second attack of tachycardia (case 2) The presence or type of organic heart disease does not seem to influence the length of the period with attacks of tachycardia (Table 3)

During the period of ventricular tachycardia 4 patients had at least one attack per month (Table 3)

DISCUSSION

Paroxysmal tachycardia is a rare condition in infancy and childhood and in these age groups the incidence of supraventricular tachycardia by far exceeds that of ventricular tachycardia (9) The outcome of the present study indicates that ventricular tachycardia is exceptionally rare in infancy but somewhat more common in childhood In accordance with earlier published case reports it can be stated that additional heart disease is the rule rather than the exception in children with paroxysmal ventricular tachycardia Thus in the present series about 60% of the children had additional heart disease The predominant aetiology of ventricular tachycardia in this series has been myocardial disease either myo

carditis or cardiomyopathy which compares very well with the case reports found in the literature

The symptomatology of paroxysmal ventricular tachycardia varies from child to child just as it does in *children* with tachycardia of supraventricular origin (9) Total absence of symptoms has in the present series been noted in one child only whereas the major part of the children complained of palpitation tiredness or weakness and some furthermore of dyspnoea precordial pain or abdominal pain Tachypnoea has been observed in 3 of the 11 cases during tachycardia signs of overt congestion—neck vein stasis hepatomegaly or pulmonary oedema—in 2 cases

The significant diagnostic ECG criteria (31) are broadened QRS complexes different in shape from the complexes during sinus rhythm (Figs 2 and 3) together with atrioventricular dissociation (Fig 2) *and/or* ventricular captures or fusion beats (Figs 2 *B* and 3 *A*) *and/or* observation of ventricular ectopics during a period of sinus rhythm with a QRS configuration similar to that observed during tachycardia Atrioventricular dissociation has been a common finding in the present series (7 out of 11 cases) Most attacks of ventricular tachycardia lasted 2 to 3 hours exceptionally several days or may be weeks In the present series 3 of the 11 children suffered from "brief malignant tachycardia a term which denotes an electrocardiographical-clinical syndrome with characteristic features (31) ECG at rest usually shows sinus rhythm but more or less heavy exercise and emotional stress initiates tachyarrhythmias in the form of bursts of ventricular ectopics which during continued stress occasionally convert into ventricular fibrillation leading to syncope or subside within few minutes in the resting state The child does not feel the brief ventricular tachycardia and his attacks of syncopes are often misdiagnosed as being due to epilepsy Consequently the diagnosis of brief malignant tachycardia has to be considered in an child with stress provoked syncopes which again means that such patients have to be investigated by continuous ECG recording during heavy exercise and if possible also by long term telemetry ECG monitoring

In the 3 patients with brief malignant tachycardia preventive treatment with a beta blocking agent reduced the incidence and severity of the attacks (35) In the remaining 8 cases the efficiency of preventive drug treatment was less convincing but at least it did seem to have reduced the frequency of attacks significantly in a couple of patients Most often the acute paroxysms of tachycardia stopped 1–2 hours after treatment but on other occasions when no treatment was given the tachycardia stopped spontaneously within the same period of time

In conclusion it could not be proved that the given antiarrhythmic treatment was of any benefit

Nowadays lidocaine not used in this series would be the drug of first choice for intravenous treatment of ventricular tachycardia (33) Next a betablocking agent may be considered (15) but the negative inotropic effect of these drugs should be kept in mind and therefore they should not be given—or at least administered very cautiously—to patients with signs of congestion In case of life threatening—or drug resistant—tachycardia d c cardioversion should always be considered (29)

One patient with cardiomyopathy in our material died 6 years after her first attack Furthermore the 3 children with brief malignant tachycardia have had repeated life threatening attacks of cardiac arrest resulting in sustained unconsciousness One of these children once had to be resuscitated by d c -countershock after several minutes of ventricular fibrillation Otherwise the prognosis seems to depend first of all on the type of additional heart disease The attacks of tachycardia subsided in about two years in half of the children in the present series whereas in one child 9 years elapsed between the first attack and the

start of a new period of attacks of years duration

SUMMARY

A retrospective 3–20 years follow up study of 1 infant and 10 children with paroxysmal ventricular tachycardia (8 cases) or fibrillation (3 cases) is presented Additional heart disease has been observed in 60 (myocarditis 3 cases cardiomyopathy 3 cases congenital heart disease 1 case) Symptoms of tachycardia were palpitation tiredness, weakness dyspnoea precordial and abdominal pain Syncopes were observed in 3 cases 1 child had no symptoms Tachycardia of hours to days duration have been noted in 8 patients, 7 had tachycardia of repetitive type with a v dissociation one continuous tachycardia without any intervening sinus beats after the onset of tachycardia and with retrograde atrial activation The remaining 3 children had brief malignant tachycardia i e stress provoked bursts of ventricular activity occasionally converting to ventricular fibrillation causing syncope *The diagnosis of brief malignant tachycardia* had to be based on exercise ECG (2 cases) or long term ECG monitoring by telemetry (one case) One child with cardiomyopathy had died at the time of follow up and the 3 patients with brief malignant tachycardia had experienced repeated life threatening attacks—one of which had to be stopped by emergency d c countershock The period of attacks ceased *in 5 cases* within 2 years after onset in 1 patient 9 years elapsed between first and second attack Preventive treatment with betablocking agents proved beneficial in the 3 children with brief malignant tachycardia

ACKNOWLEDGEMENT

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Key words Paroxysmal tachycardia ventricular tachycardia, fibrillation infants children

start of a new period of attacks of years duration

SUMMARY

A retrospective 3–20-years follow up study of 1 infant and 10 children with paroxysmal ventricular tachycardia (8 cases) or fibrillation (3 cases) is presented Additional heart disease has been observed in 60% (myocarditis 3 cases cardiomyopathy 3 cases congenital heart disease 1 case) Symptoms of tachycardia were palpitation tiredness weakness dyspnoea precordial and abdominal pain Syncopes were observed in 3 cases 1 child had no symptoms Tachycardia of hours to days duration have been noted in 8 patients 7 had tachycardia of repetitive type with a v dissociation one continuous tachycardia without any intervening sinus beats after the onset of tachycardia and with retrograde atrial activation The remaining 3 children had brief malignant tachycardia i e stress provoked bursts of ventricular activity occasionally converting to ventricular fibrillation causing syncope The diagnosis of brief malignant tachycardia had to be based on exercise ECG (2 cases) or long term ECG monitoring by telemetry (one case) One child with cardiomyopathy had died at the time of follow up and the 3 patients with brief malignant tachycardia had experienced repeated life threatening attacks—one of which had to be stopped by emergency d c countershock The period of attacks ceased in 5 cases within 2 years after onset, in 1 patient 9 years elapsed between first and second attack Preventive treatment with betablocking agents proved beneficial in the 3 children with brief malignant tachycardia

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Table 1 *Social class distribution of continuous and discontinuous soilers*

Type of soiling	I	II	III	IV	V	Total
	Occupational class					
Continuous	—	—	1	—	4	5
Discontinuous	—	1	9	5	3	18
Total	—	1	10	5	7	23

In 7 cases the occupation of the father was not known

community studied by Bellman (2) and between the encopretic clinic attenders and other disturbed children attending the same department

METHOD

The referral register was scrutinised and the records of every child whose referral symptoms included encopresis were selected for closer study The period under review covered twelve months Of 530 children referred to the Department of Psychological Medicine Royal Hospital for Sick Children Edinburgh during this time 33 came with a complaint of encopresis although occasionally this was not the main or only symptom One child was excluded from the study because the father did not think soiling was a problem for a psychiatrist and no history was volunteered The 3 remaining children who form the subjects of this study included two pairs of brothers Thirty families were involved

The information abstracted from th case notes included the age and sex of the child source of referral occupation of father size of sibship factors associated with the onset of the soiling type of soiling, and the child's intelligence Only limited use can be made of case notes in which information is not uniformly recorded Rating scales were devised for assessing certain cleaned features on the basis of the case records The features were the child's level of anxiety at interview his associated symptoms using Rutter's classification (14) the mother's toilet training method the social competence of the family (For details see Appendix A) The author and another child psychiatrist independently rated the subjects on these variables to establish the reliability of the scales Correlations were calculated using Kendall's tau method (9) Fairly good agreement was found between the two raters although the case notes varied greatly in the amount of detail recorded Kendall's tau was between 0 43 and 0 67 $p < 0\ 001$ for all the scales The final ratings used were the author's

Table 2 *Social competence and type of soiling*

Rating of social competence	Continuous	Discontinuous	Total
Good	—	5	5
Average	2	4	6
Poor	4	17	21
Total	6	26	32

RESULTS

The prevalence of encopresis

The encopretic children referred to the child psychiatric clinic formed 5 7% of the total number of referrals during the year Vaughan & Cashmore (17) reported a series which constituted 10 of the total number of children seen over a 2 year period in a child psychiatric department Encopretic children attending a psychiatric department are highly selected since many children are seen by general practitioners and paediatricians without subsequent referral to a psychiatrist Bellman (2) found the prevalence of encopresis at least once a month among 7 year-old primary school children in Stockholm to be 1 5

Age at referral

The mean age of the children at the time of referral was 7 66 years (range 3–13 years) All the children were of pre school or primary school age The fact that only 4 children were 11 years or over supports the view that most soilers overcome their difficulty by adolescence

Sex

The sex ratio of 25 boys to 7 girls i e 3 5 1 is in accord with previously reported series (1 2 10)

Type of encopresis

Of the 32 encopretic children studied 26 were discontinuous soilers and only 6 had never had bowel control

Social class

The Registrar General's classification of occupations was adopted in this study (8) Table 1

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ENCOPRESIS

A Review of Thirty two Cases

M O OLATAWURA

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Since Weissenberg (18) coined the term en copresis for faecal soiling in children various authors have proposed other labels with aetiological implications Richmond et al (13) sug gested idiopathic or psychogenic megacolon Browne (4) suggested colonic inertia while Berg & Jones (3) called the disorder func tional incontinence

A variety of explanations for the condition have been put forward based on psychological, psychoanalytic somatic psychosomatic and behaviourist theories (1 2 5 6 11 13 16)

Different types of encopresis have been de scribed In an intensive study of 76 cases of encopresis Anthony (1) recognised three types Firstly he described the continuous soiler whose symptom is continuous with the train ing period Such a soiler is aggressive dis inhibited overactive and shameless He is fur ther distinguishable by having a mother who is lax in her training practices who belongs to the lowest socio economic class who is dirty and has a high tolerance of symptoms Second ly Anthony described a discontinuous type who was once successfully toilet trained but started soiling in response to later stress He is neurotic, inhibited and very ashamed of his soiling He comes from a rigid, compulsive family usually of middle or upper socio eco nomic status His mother uses coercive training methods The third variety called the reten tive soiler could go for days without opening his bowels He too had often received coercive toilet training

Easson (7) produced a different sub classi fication He labelled Anthony's continuous type primary infantile encopresis the dis continuous type 'secondary infantile enco presis he called the continuous type with re tentive episodes primary reactive encopresis and the discontinuous type with retention sec ondary reactive encopresis Berg & Jones (3) proposed yet another sub classification using the presence or absence of constipation as the main criterion Anthony's classification had the merit of simplicity and easy application It also promised to be of greatest clinical rel evance Discontinuous neurotic soilers re quired psychotherapy while continuous, in adequately trained soilers required systematic training

AIMS

This study set out to test the usefulness of Anthony's sub classification of encopresis The records of all encopretic children referred to a child psychiatry department in one year were studied The characteristics of the children and their families were compared with those of the children described by Anthony In addition comparisons were made between this group of clinic attenders and encopretic children in the

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This study formed the basis of a dissertation for the Edinburgh University D P M

Table 1. Social class distribution of continuous and discontinuous soilers

Type of soiling	Occupational class I	II	III	IV	V	Total
Continuous	—	—	1	—	4	5
Discontinuous	—	1	9	5	3	18
Total	—	1	10	5	7	23*

*In 7 cases the occupation of the father was not known.

community studied by Bellman (2) and between the encopretic clinic attenders and other disturbed children attending the same department.

METHOD

The referral register was scrutinized and the records of every child whose referral symptoms included encopresis were selected for closer study. The period under review covered twelve months. Of [illegible] children referred to the Department of Psychological Medicine, Royal Hospital for Sick Children, Edinburgh during that time, 33 cases with a complaint of encopresis, although occasionally this was not the main or only symptom. One child was excluded from the study because the father did not think soiling was a problem for a psychiatrist and no history was volunteered. The 32 remaining children who form the subjects of this study included two pairs of brothers. Thirty families were involved.

The information abstracted from the case notes included the age and sex of the child, source of referral, occupation of father, rate of soiling, factors associated with the onset of the soiling, type of soiling and the child's intelligence. Only limited use can be made of case notes in which information is not uniformly recorded. Rating scales were devised for assessing certain clinical features on the basis of the case records. The features were: the child's level of anxiety at interview, his associated symptoms using Rutter's classification (14), the mother's toilet training method, the social competence of the family. (For details see Appendix A). The author and another child psychiatrist independently rated the subjects on these variables to establish the reliability of the scales. Correlations were calculated using Kendall's tau method (9). Fairly good agreement was found between the two raters although the case notes varied greatly in the amount of detail recorded. Kendall's tau was between 0.43 and 0.67, $p < 0.001$ for all the scales. The final ratings used were the author's.

Table 2. Social competence and type of soiling

Rating of social competence	Continuous	Dis-continuous	Total
Good	—	5	5
Average	2	4	6
Poor	4	17	21
Total	6	26	32

RESULTS

The prevalence of encopresis

The encopretic children referred to the child psychiatric clinic formed 5.7% of the total number of referrals during the year. Vaughan & Cashmore (17) reported a series which constituted 10% of the total number of children seen over a 2 year period in a child psychiatric department. Encopretic children attending a psychiatric department are highly selected since many children are seen by general practitioners and paediatricians without subsequent referral to a psychiatrist. Bellman (2) found the prevalence of encopresis at least once a month among 7 year-old primary school children in Stockholm to be 1.5%.

Age at referral

The mean age of the children at the time of referral was 7.66 years (range 3–13 years). All the children were of pre-school or primary school age. The fact that only 4 children were 11 years or over supports the view that most soilers overcome their difficulty by adolescence.

Sex

The sex ratio of 25 boys to 7 girls, i.e. 3.5 : 1 is in accord with previously reported series (1, 2, 10).

Type of encopresis

Of the 32 encopretic children studied, 26 were discontinuous soilers and only 6 had never had bowel control.

Social class

The Registrar General's classification of occupations was adopted in this study (8). Table 1

shows the distribution of fathers occupational class for continuous and discontinuous soilers

In the seven cases in which the occupation of the father was unknown other indices of social circumstances suggested that nearly all were likely to hold labouring or semi skilled jobs

Encopretic children thus came predominantly from lower social class families They differed from child psychiatric clinic attenders as a whole whose social class distribution is the same as that of the general population (19)

Anthony s finding that discontinuous soilers came mainly from middle class or skilled working class homes was not confirmed but there is a suggestion that continuous soilers come from an even poorer socio economic background

Social competence of family and type of soiling

Anthony s description of the continuous soilers suggested that they come from socially incompetent families while discontinuous soilers in his series came from well organised homes The relationship between the type of soiling and the social competence of the family in the present series is presented in Table 2

As many as 17 of the 26 discontinuous soilers were rated as coming from socially incompetent families This is contrary to Anthony s suggestion that discontinuous soilers come from compulsive socially competent families

Intelligence

Table 3 shows that the 20 children who had been tested psychologically had a somewhat low range of intelligence Their test results were lower than those of other children attending the same department who were referred by the school psychological service (12) The range of intelligence of Bellman s series of encopretic children was similar to that of a matched control group of children and 75 7% were in the normal range of 85–115

Table 3 *Distribution of intelligence*

IQ	60–80	81–100	101–120	120	Total
Number of children	5	8	5	2	20*

* 12 children had not been tested

Evidence of neurological impairment and developmental disorders

Six of the encopretic children had evidence suggestive of brain damage (neurological abnormality abnormal EEG epileptic fits psychiatrist s diagnosis of hyperkinetic syndrome or developmental speech defect Hyperactivity alone was not considered a neurological disorder) This is a high proportion for this clinic It had previously been found that only 3 out of 103 consecutive primary school children referred had a diagnosis of minimal brain damage (19)

Rutter (14) advocated that monosymptomatic enuresis should be classified as a developmental disorder Nineteen encopretic children were also enuretic and of these 9 children had no other symptoms and could be classified as having a developmental disorder In addition 3 other encopretic children had no associated symptoms whatever

Associated enuresis

In the course of normal child development bowel control is usually achieved earlier than bladder control One might expect continuous soilers to have associated enuresis more often than discontinuous soilers on the basis that continuous soiling is more likely to be due to a developmental lag or to inadequate training Anthony (1) too found discontinuous encopretics to be more often monosymptomatic than continuous encopretics In the present series however 17 of the 24 discontinuous soilers were also enuretic while only two of the six continuous soilers had associated enuresis

Enuresis was more often associated with encopresis in girls (5 out of 7) than in boys (14 out of 25)

Table 4 *Anxiety level and types of soiling*

Anxiety level	Continuous	Dis continuous	Total
Definitely anxious	2	10	12
Possibly anxious	2	11	13
Not anxious	2	5	7
Total	6	26	32

Events associated with onset of encopresis

The precipitants mentioned by the parents of the 26 discontinuous soilers were as follows starting nursery school in 7 hospital admission of parent or child in 3 fissure in ano in 2 mother starting to go out to work in 1 car accident in 1 birth of a new baby in 1 prolonged family stress in 10 unknown in 1 Prolonged family stress included parental fights father s aggressiveness when drunk desertion of mother remarriage of mother after death of father and parental separation

Prolonged family stress was by far the commonest precipitant and as in Bellman s study (2) fissure in ano was rare In Bellman s study emotional participants were common in encopretic children In the present study they were identified in 88 5% of cases

Other associated symptoms

Vaughan & Cashmore (17) observed that soiling is only one of the complaints that bring the encopretic child to a child guidance clinic Anthony (1) in describing his two main types of soilers also pointed out that soiling may not be the only symptom of the encopretic child In the present sample soiling was usually one of the presenting symptoms The children s associated symptoms were classified according to Rutter s diagnostic scheme (14) and were found to be as follows neurotic behaviour disorder 9 conduct disorder 6 mixed behaviour disorder 5 developmental disorder 9 no associated symptoms 3 Thus there were more neurotic and developmental than antisocial disorders despite the preponderance of boys and the low social class of the children In a child guidance population boys are generally more antisocial than girls especially if they come predominantly from lower social class homes as in this sample

Anxiety level in relation to type of soiling

Anthony (1) described his continuous type of soiler as overactive and disinhibited showing no anxiety about his soiling while the discontinuous soiler was inhibited anxious and ashamed of his soiling The level of anxiety was the one characteristic of the child which was thought to be more uniformly mentioned in the records It was decided to see whether the continuous and discontinuous type of soilers differed on the level of anxiety shown at the first interview (Table 4)

The small numbers in this sample do not permit within group comparison with respect to the level of anxiety Table 4 however shows that 25 of the 32 children were anxious at the first interview Since this sample consists predominantly of discontinuous soilers Anthony s finding that these are anxious children is borne out

Family disruption

Rutter (15) has shown that children seen at the Maudsley Hospital Children s Department came more often from broken homes than control groups of children attending paediatric and dental clinics

In the present study 14 of the 30 families (46 6) from which the children came were disrupted This is considerably higher than the rate of broken homes (28) of a series of primary school children referred to the same child psychiatric clinic (21)

Personality disorder of mother

Ruttar (15) found that a third of the parents of psychiatrically ill children had received a diagnosis of personality disorder compared with one in five of a control group Personality disorder is an abnormality associated with difficulties in interpersonal relationships It might therefore be supposed that if stressful e g coercive toilet training methods are important

in the aetiology of encopresis, many mothers of encopretic children would have recognisable personality disorders The scale used by Wolff & Acton (21) in their study of parents of Edinburgh primary school children was used to rate the descriptions of mothers found in the same records Fifteen mothers were rated as having a mild personality disorder, seven as having a definite personality disorder four as "sociopathic and four as having no personality disorder Wolff & Acton found that mild personality disorder did not differentiate mothers of clinic attenders from mothers of a control group of children Fifty one per cent of mothers of children attending the clinic with behaviour disorders had definite or sociopathic personality disorders This compares with only 36 7% in the present series

Mother s training method and type of soiling

Anthony (1) found that discontinuous soilers tend to have coercive mothers and continuous soilers mothers lax in toilet training The relationship between type of soiling and the mother s training method is shown in Table 5

It was impossible to rate the type of training nine of the children had received The finding that 13 of the remaining 23 soilers had been subjected to relatively normal training pressure suggests that laxity and coercion of training cannot explain the genesis of soiling

From this sample all that can be said about Anthony s findings concerns the discontinuous group because the continuous soilers were too rare The discontinuous soilers were not found to have particularly coercive mothers

Table 5 *Training method and type of soiling*

Training method	Continuous	Dis continuous	Total
Coercive	1	2	3
Average	1	12	13
Lax	1	6	7
Not known	3	6	9
Total	6	26	32

DISCUSSION

The present series of children is numerically small and the study is based on case records Moreover the fact that the majority of the children are below the age of 11 years is another source of difficulty when they are divided into different groups of diagnosis However certain clear cut findings emerged from this study

Unlike encopretic children in the community (2) encopretic children referred to a psychiatric department who were as a rule persistent and severe soilers, came predominantly from low socio economic backgrounds and contained an excessive number of intellectually dull children Moreover more children in this small series (six) had evidence of neurological impairment than did other children attending the same department Encopresis was monosymptomatic in 3 children and associated with enuresis as the only other symptom in 9 These 12 children could be regarded as having a developmental disorder There were two pairs of siblings

Since 26 of the 32 children were discontinuous soilers (1) and only 6 had never achieved toilet control this study provides more information about the former type of encopretic than about the latter continuous type which is rare in this series The discontinuous soilers in the present series differed from Anthony s in that they came predominantly from semi and unskilled working class families lacking in social competence There was no evidence that these discontinuous soilers had been toilet trained coercively

On the other hand as Anthony (1) had suggested they were as a group anxious children whose associated behaviour disorders were classified as neurotic or developmental more often than as conduct or mixed disorders This was the more striking in view of the poor social background of the children and the predominance of boys among them

Stressful events preceded the onset of soiling in 23 of the 26 discontinuous soilers and con-

sisted most commonly of prolonged family stress often the result of marital discord A higher proportion of children in this small series were found to have disrupted families than in the clinic population as a whole Yet the mothers of the encopretics were not as personality disordered as the mothers of other clinic attenders

The *findings of this study suggest that en* copresis in children who attend a psychiatric department generally arose on the basis of ex cessive anxiety resulting from stressful life ex periences Poverty and social incompetence of the family rather than coercive toilet training formed one set of predisposing factors Devel opmental lags at times associated with neuro logical deficits and low intelligence consti tuted another

SUMMARY

Thirty two children attending a child psychi atric department with encopresis are described Twenty six were discontinuous and only 6 were *continuous soilers*

As a group they came from low socio-economic socially incompetent families and a number of children had evidence of develop mental lags or neurological impairment Coer cive toilet training featured very rarely in the case histories

Twenty three of the 26 discontinuous soilers had experienced stressful events prior to the onset of the symptom and many came from disrupted families

They were as a group anxious children whose associated symptoms fell into the cate gories of neurotic or developmental disorders rather than of conduct or mixed disorders

ACKNOWLEDGEMENTS

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Total	6	26	3?

Unlike encopretic children in the community (2) encopretic children referred to a psychiatric department, who were as a rule persistent and severe soilers came predominantly from low socio-economic backgrounds and contained an excessive number of intellectually dull children Moreover more children in this small series (six) had evidence of neurological impairment than did other children attending the same department Encopresis was monosymptomatic in 3 children and associated with enuresis as the only other symptom in 9 These 12 children could be regarded as having a developmental disorder There were two pairs of siblings

Since 26 of the 32 children were discontinuous soilers (1) and only 6 had never achieved toilet control, this study provides more information about the former type of encopretic than about the latter continuous type which is rare in this series The discontinuous soilers in the present series differed from Anthony s in that they came predominantly from semi and unskilled working class families lacking in social competence There was no evidence that these discontinuous soilers had been toilet trained coercively

On the other hand as Anthony (1) had suggested they were as a group anxious children whose associated behaviour disorders were classified as neurotic or developmental more often than as conduct or mixed disorders This was the more striking in view of the poor social background of the children and the predominance of boys among them

Stressful events preceded the onset of soiling in 23 of the 26 discontinuous soilers and con

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UNUSUAL COMBINED IMMUNODEFICIENCY SYNDROME EXHIBITING KAPPA IGD PARAPROTEINEMIA RESIDUAL GUT IMMUNITY AND GRAFT VERSUS-HOST REACTION AFTER PLASMA INFUSION[1]

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Since Bruton's discovery of agammaglobulinemia a variety of immune disorders have been described The patient reported here may be classified under the variable type of severe combined immunedeficiencies (CID) (11) with the exception of two unique findings (*a*) Apparently normal IgA in the gut in contrast to its absence in other external fluids (*b*) A kappa IgD paraproteinemia

CASE REPORT

A 12 year-old boy from a normal sibship was first seen at the University Hospital of Berne in 1965 with the complaint of chronic poor health Since the age of 10 days he had had watery diarrhea Conjunctivitis started soon after birth Whooping cough German measles, and varicella took serious almost fatal courses (Fig 1) Bouts of bacterial septicemia became more frequent despite antimicrobial therapy including weekly intravenous administration of 30 ml 6% gammaglobulin (SRK PH-4 gammaglobulin) In addition, candida was isolated on two occasions from skin and oral lesions At the age of 11 years during a therapeutic trial with fresh plasma infusions, a temporary improvement was followed by an acute deterioration with clinical signs characteristic of an acute graft versus host reaction (GvHR) These manifestations included weight loss an exfoliative enteropathy a scaly skin rash which persisted for 3 months and a hepatomegaly with concomitant elevation of serum transaminases During this episode abundant histiocytes, foam cells and atypical lymphomonocytes became increasingly detectable in the bone marrow Yet there was no evidence of a XX/XY chimerism nor were additional HL A antigens demonstrable However a transient significant increase of serum IgM was observed (Fig 3) Most of the signs of GvHR except for the diarrhea and the hepatitis subsided at the age of 12 years when this patient received an HL A compatible bone marrow graft resulting in an almost complete immunological reconstitution (32) Prior to the transplantation the patient was markedly dystrophic his bodyweight was only 16 kg and his growth stagnated at the height of 124 cm There was dyspnea and cyanosis at rest and prominent clubbing of the fingers Moist rales were heard over the entire lung fields The liver edge was palpated _ cm below the right costal margin No spleen and no lymph nodes were palpable tonsils were not visible His hemoglobin was 8 4 g per 100 ml the white cell count 2 800 per cmm with a predominance of toxic neutrophils (78%) The lymphocyte count was 140 per cmm rare plasma cells occurred in the peripheral blood The bone marrow showed severe depletion of lymphocytes the myelopoiesis was characterized by a shift to the left and toxicity Abundant large immature myelocytes reminiscent of preleukemic forms were observed

STUDIES OF THE IMMUNE RESPONSE

Methods

Serum immunoglobulins were measured by radial immunodiffusion using specific antisera according to the method of Mancini (20) Lowest standard for IgA was 0 3 mg per 100 ml Kappa and lambda selection plates for

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APPENDIX A

Rating Scales

Name

1 Child s level of anxiety at interview

(a) Definitely anxious 3
(b) Possibly anxious 2
(c) Not anxious 1

2 Child s associated symptoms

(a) Neurotic behaviour disorder 1
(b) Conduct disorder 2
(c) Mixed behaviour disorder 3
(d) Developmental disorder 4
(e) None 5

3 Mother s training method

(a) Coercive 3
(b) Average 2
(c) Lax 1

4 Personality disorder of mother

(a) None 1
(b) Mildly abnormal i e character disorder (dependent aggressive inhibited or detached) 2
(c) Definitely abnormal personality (obsessional hysterical paranoid schizoid cyclothymic) 3
(d) Sociopathy (include here aggressive and inadequate personalities alcoholism promiscuity perversions delinquency) 4

5 Social competence of family (as described by Psychiatric Social Worker) (include here excessive drinking in father or mother evictions debts fighting)

(a) Good 1
(b) Average 2
(c) Poor 3

6 Suggestive evidence of brain damage

C N S abnormality found on neurological examination
Abnormal E E G
History of epileptic fits
Hyperkinetic syndrome associated with signs of neurological damage
Speech defect

Table 1 *Serum immunoglobulins mg/100 ml*
(Without gammaglobulin therapy and before infusions of fresh plasma)

	1965		1969			Normal values
IgA	0	0	0	0	0	77–257
IgG	575	700	820	680	245	852–1536
IgM	39	13	13 5	12	12	38–190
IgD				60	62	0 3–30

stimulation with phytohemagglutinin (PHA) and with allogeneic lymphocytes according to Bach & Voynow (2) Serum lymphocytotoxic antibodies were detected by the Terasaki method (36) The macrophage migration inhibition assay was performed in the indirect test by the technique of Thor et al (37) and in the direct test according to Søborg (35) Duodenal and rectal biopsies were stained with fluorescent FITC antisera to IgA IgG and IgM according to Rubin et al (31) Albumin half life was determined by the assay of Waldmann & Strober (40)

Results

Serum IgA as well as anti IgA agglutinants were repeatedly absent IgM was markedly diminished while IgG was only slightly reduced However IgD was significantly elevated (Table 1) By the use of light chain selection plates (Fig 2) an IgD paraprotein of kappa type could be demonstrated IgA was absent in parotid saliva in the urine and in the tears Only traces of IgA were detectable in the stools In the duodenal fluid IgA mounted to 17 5 mg per 100 ml The secretory component was present in all specimen of external fluids *including those lacking secretory IgA* No specific serum antibodies were found after infection with German measles nor after three vaccinations with tetanus toxoid In spite of streptococcal infections no antistreptolysin was detectable in the serum The patient's blood group was A but serum anti B isohemagglutinins were absent Normal neutralizing antibodies were found in response to oral live poliomyelitis vaccine No thymic shadow could be demonstrated by tomography X ray after pneumomediastinum Lymph angiography revealed atrophic inguinal iliac and aortic lymph nodes After tetanus immunizations a very small regional lymph node showed severe depletion of lymphoid cells and an absence of typical germinal centers and plasma cells The sinusoids and the medullary cords were well preserved and the network of reticulum cells in some regions

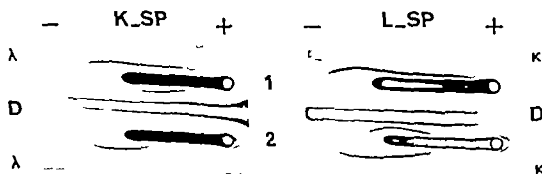


Fig 2 Light chain typing by immunoselection plates for IgD In the corresponding selection plate (K SP and L SP) are anti K and anti L light chain antisera incorporated in the agar The troughs contain specific antiserum against IgD In the holes normal human serum containing an increased IgD level of 40 mg/100 ml (1) is run electrophoretically in comparison to the patient's serum (2) While in the normal serum the IgD is mostly of lambda (L) type and shows electrophoretic heterogeneity in the patient's serum the IgD (62 mg%) is only of the kappa (K) type and the precipitin line indicates an electrophoretic homogeneity

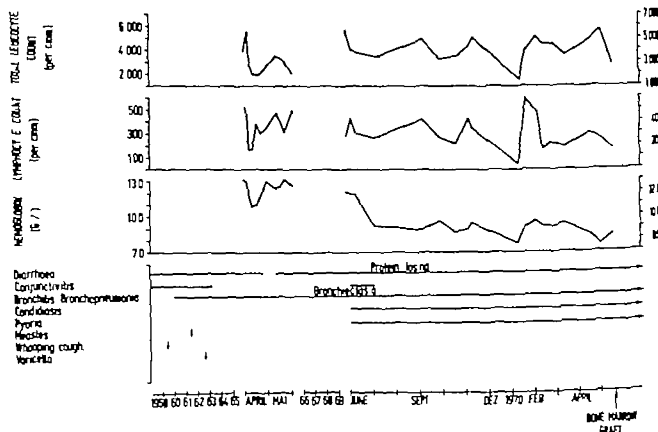


Fig 1 Relevant data from the patients case history

light chain typing were performed according to Radl (28) Parotid saliva was collected under stimulation with citric acid for 30 minutes using special cups The specimens were dialysed and freeze dried IgA was measured by radial immunodiffusion in 30–40 times concentrated saliva IgA in the 24 hour urine was concentrated 1 000 times and measured according to Bienenstock & Tomasi (3) Stool IgA was extracted by the method of Kawakami et al (17) Duodenal juice was assayed for immunoglobulins by radial immunodiffusion either immediately or after dialysis at 4 C Overspilled tears were collected in capillaries after stimulation with onions and assayed for IgA by radial immunodiffusion and by electroimmunodiffusion The further preparation of antisera to IgA and to secretory component were adapted from Newcomb et al (23) External (exocrine) IgA was isolated from defatted human colostrum on Sephadex G 200 and by ion exchange chromatography on DEAE cellulose Purified external IgA was injected in complete Freund adjuvant into goats Antibodies to colostral IgA were absorbed with IgA free cord blood and with lactoferrin They showed a dual specificity for IgA and for secretory component in the immunoelectrophoresis and in double diffusion in agar gel After absorption with an excess of normal serum IgA a monospecificity for secretory component was achieved Semiquantification of the secretory component was performed by double diffusion in agar gel Neutralizing antibodies in response to poliomyelitis vaccination were determined according to Lennet & Schmidt (18) Antibodies to measles were measured by complement fixation and to tetanus by the hemagglutination test according to Pittman et al (25) Skin tests for delayed type hypersensitivity including dinitrochlorobenzene (DNCB) sensitization were performed in accordance with the WHO recommendations (11) *In vitro* reactivity of lymphocytes was measured in triplicate by the incorporation of tritiated thymidine after

monoclonal immunoglobulins have rarely been noted in children suffering from immunodeficiencies (26) except for limited periods af ter hematopoietic cell grafts (9 27 32) In accordance with these findings our patients IgD paraprotein disappeared after a successful bone marrow transplantation but at the same time other proteins of restricted heterogeneity such as kappa IgM and kappa IgG_1 became transiently detectable (32) The second as sumption mentioned above is also inconceiv able since such a profound failure of antibody formation as presented here is absent in sys tems known to be deficient of T-cells namely in neonatally thymectomized animals and in patients with the DiGeorge syndrome As for the accessory cell function a defect of the macrophages was demonstrated by the macrophage migration tests However this defect did not express itself in the processing of live attenuated poliomyelitis virus introduced to the gut associated lymphoepithelial structures All these findings question the hypothesis of a solitary critical B-cell inductive structure in the gut Moreover the presented dissociation in the secretory IgA system itself raises the possibility of a heterogeneic development of this system in which the gut lamina propria comprises an independent structure The pres ence of normal jejunal IgA in a patient with deficient serum IgA was also reported by Hazenberg et al (14)

The etiology of the immune defect in our patient is still obscure The question remains —are we dealing with a primary developmental failure or with a selective lesion of the hematopoietic stem cells leaving the gut lymphoepithelial structures intact? The struc ture of the lymph node with well preserved sinusoids and medullary cords represents strong evidence for the latter hypothesis This histological picture stands in marked contrast to the usual findings in the Swiss type of agammaglobulinemia where the lymph nodes are composed of densely packed reticulum cells and fibrocytes and where lymph sinuses are often completely absent (8) Furthermore in contrast to the regularly fatal GvHR after introduction of allogeneic hematopoietic cells in patients with the primary severe CID (4 10 12 15 16 21 29 30 38) our patient seemed to recover spontaneously from this reaction His capability to cope at least tem porarily with unmatched hemopoietic grafts may be attributed to the extremely low cel lularity of the grafts and to the fact that his cellular immune response was not totally lacking Although he was unable to adequately reject an allogeneic skin graft and lacked posi tive skin tests of the delayed type hypersensi tivity the PHA reactivity of his peripheral lymphocytes was not completely absent In a retrospective study of similarly prepared plasma as that administered to our patient it was found to contain up to 8×10^4 cells per kg bodyweight which is much less than the amount assumed to be capable of inducing a fatal GvHR in CID (39) Although the GvHR was relatively mild in our patient the prognosis could still be grave since Cashman et al reported a fatal outcome of GvHR 6 years after the initial blood transfusion (4) This in dicates that a safe dose of unmatched hemopoietic cells cannot be calculated with cer tainty for patients with severe CID who are extremely susceptible to GvHR

The previously reported sources of immuno competent cells inducing GvHR have been blood bone marrow (4 10 12 13 15 21 29 38) or materno-fetal cell transfer as proven by the finding of XX/XY chimerism (16)

Since our patient has never received blood transfusions the only plausible source for al logeneic cells was fresh plasma infusions Ad ministration of fresh plasma was followed in this patient by the whole spectrum of clinical signs characteristic of GvHR namely weight loss exfoliative enteritis exfoliative derma titis and hepatitis Most characteristic was the abundance in the bone marrow of histiocytes and of atypical lymphocytes as also reported in animal experiments (30 34 39) and in man (4 10 12 13 14 16 21 29 38) after trans fusions of incompatible hematopoietic cells

Table 2 *IgA in the external fluids mg/100 ml*

	Patient	Normal values
Parotid saliva	0	0 14–0 69
Total saliva	0	4 4–7 6
Tears	0	2 9–19
Urine (24 hrs)	0	0 12–0 55
Duodenal juice	8 75–17 5	15 5–43 5
Feces	Traces	

Checked for each external fluid in at least 4 different specimens from the patient
Normal values for the age of 9–11 years measured in 35 normal individuals

was loose In various specimens of duodenal and jejunal fluids no lymphocytes were de tectable The albumin half life was shortened to 4 4 days compared with the normal half life of 13 to 20 days However no lymph angiectasia was present in duodenal and rectal biopsies The mucosa of these organs was atrophic with abundant plasma cells and lym phocytes being detectable These plasma cells stained positive with fluorescent anti IgA anti IgM and to a lesser extent with anti IgG Delayed type hypersensitivity skin reac tions to DNCB to candidin and to PPD were absent A full thickness skin allograft from an unrelated donor gave no evidence of rejec tion In vitro reactivity of peripheral lympho cytes to PHA was very low (1 10 of normal controls) HL A typing revealed identity with two siblings At no time was there evidence of the presence of additional HL A antigens of an XX/XY chimerism or of a serum lympho cytotoxin In the macrophage migration in hibition assays the patient s peripheral leuko cytes were unable to migrate even in the ab sence of antigens The supernatant of the patient s lymphocytes cultured together with PPD did not inhibit significantly the migra tion of guinea pig macrophages

DISCUSSION

The patient described here exhibited a severe CID with an exceptionally protracted clinical course (Fig 1) His longevity may be at tributable to the residual immunity com prised of moderately low serum IgG (Fig 2 Table 1) and apparently normal gut immunity In spite of the presence of serum IgG no specific antibodies were formed in response to a variety of antigenic stimuli In another re ported patient with normal serum immuno globulin levels the failure to produce specific antibodies including neutralizing antibodies to poliovaccination was associated with a scarcity of gut lymphoid tissue (33) This does not apply to our patient in whom evidence for an at least partially functioning gut associated lymphoid structure was obtained Although secretory IgA was absent in the saliva in the tears and in the urine it was demonstrated in the stool and in the duodenal juice (Table 2) Correspondingly histological and immuno fluorescence studies of rectal and duodenal biopsies revealed abundant IgA positive cells and to a lesser extent cells with cytoplasmatic IgM and IgG Moreover despite a lack of immune response to tetanus immunizations neutralizing antibodies were formed normally in response to orally administered live polio myelitis vaccine In the latter instance the alimentary tract is known to play an essential role giving rise to coproantibodies as well as to circulating antibodies (19 24) These find ings further emphasize the problem of localiza tion of the avian bursal equivalent in man (1 5–7) Among others it has been postulated that the differentiation of stem cells to plasma cells requires their migration to gut associated lymphoepithelial tissues (5–7) If this gut asso ciated structure is assumed to be critical for B cell induction the failure to elicit an ade quate humoral immune response in lieu of the apparently normal inductive system in the pa tient described here could only be attributed to (*a*) lack of further presursors of B-cells (*b*) an inefficient interaction between the B and T cell system (22) (*c*) a defect in the path way of processing antigens namely in the ac cessory cell function

The first assumption is unacceptable in the presence of intestinal IgA of serum IgG and of an elevated serum kappa IgD Apparently

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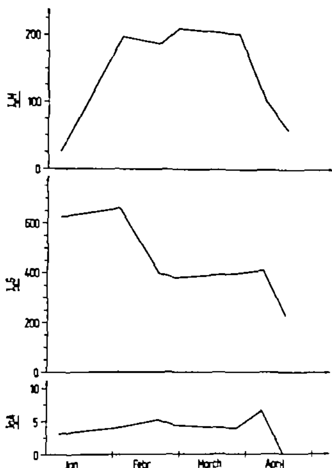


Fig 3 Serum immunoglobulins (mg/100 ml) during accidental engraftment

Although we could not prove chimerism the transient detection of significantly increasing serum IgM levels (Fig 3) during the GvHR implies an accidental engraftment Unfortunately there were no plasma donors available for the study of the genetic markers of the immunoglobulins However the observation of augmenting serum IgM levels corresponds to the initial reconstitution of IgM synthesis in CID after successful hemopoietic grafts (9 27 32) In our patient the same mode of immunoglobulin reconstitution followed a transplant of compatible bone marrow (32) The proof of an at least temporary accidental engraftment after the introduction of only 8×10^4 cells per kg may further indicate that extremely low cellular hemopoietic grafts could be attempted also in the typical severe CID thus reducing the hazard of an acute fatal GvHR

SUMMARY

A 12 year old suffering from a lymphopenic severe CID with an unusual protracted clinical course is presented His gut associated lymphoepithelial system was apparently normal in contrast to the IgA deficiency in other external fluids In addition an elevated kappa IgD was detected in his serum Fresh plasma infusions from unrelated donors induced an accidental engraftment with a moderate non fatal GvHR

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MALOCCLUSION AND SUCKING HABITS OF FOUR YEAR OLD CHILDREN

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Malocclusion in the deciduous dentition is common ranking third as oral disorder after caries and gingivitis (5 7 9 20, 26) Sucking habits in children are prevalent in all communities and also cause of widespread concern among parents and professionals (17)

The aim of the present study is to report on the prevalence of malocclusion in an unselected population of 4 year-old children and to elucidate the connection between the findings of malocclusion and the children's past and present sucking habits The investigation is part of a general health control of 4-year old children in the city of Lund and in the community of Dalby both located in the southern part of Sweden (11–16)

MATERIAL

All children of 4 years of age living in Lund and Dalby were selected from the county population register There was a total of 1 736 4 year-old children 1 459 living in Lund 1967–68 and 277 in Dalby 1968–69

METHODS

The children were invited to participate by a letter to their parents The dental examinations took place in the Department of Public Dentistry in Lund usually 1 3 weeks after the medical examination in Dalby on the same day as the medical examination and were performed by the same dentist (K. H.)

Caries and gingivitis were examined clinically with mirrors and probes and as a rule with two posterior bitewing radiographs Caries and restorations were recorded for each tooth surface and registered as defs index (decayed extracted and filled surfaces) Gingivitis index was recorded for second primary molars and primary incisors (12 teeth) Details of the caries and gingivitis recordings are given elsewhere (15)

The registration of malocclusion was made according to the method described by Björk et al (3) and was divided into 4 parts

A *Anomalies of the dentition* i e tooth anomalies abnormal eruption and malalignment of individual teeth

B *Occlusal anomalies* i e deviation of the positional relationship between the upper and lower dental arches

(*a*) *Sagittal* Postnormal (distal) bite i e the tip of the mesio-buccal cusp of the second deciduous maxillary molar occludes on one or both sides mesial to the tip of the mesio-buccal cusp of the second deciduous mandibular molar In case of uncertainty the bite was recorded as postnormal if the central axis of the maxillary cusp lay mesial to that of the mandibular cusp on one or both sides Prenormal (mesial) bite i e the tip of the mesio buccal cusp of the second deciduous maxillary molar occludes on one or both sides in the distal of the two buccal grooves of the second deciduous mandibular molar

Maxillary overjet > 3 mm Mandibular overjet > 0 mm

(*b*) *Vertical* Open bite frontal > 0 mm Open bite lateral > 0 mm Deep bite frontal > 2 mm

(*c*) *Transverse* Crossbite uni or bilateral Scissors bite uni or bilateral

C *Deviations of space conditions* i e crowding or abnormal spacing of the teeth

D *Actual need of treatment* (one or more groups may be recorded) 1 Grinding of teeth 2 Extraction 3 Appliance therapy 4 Oral surgery

The form used for registration of malocclusion is shown in Fig 1

Interview

Immediately after the dental examination the mother was interviewed regarding the child's previous and

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Table 1 *Malocclusions in 1567 four year old children*

	Lund (n = 1313)		Dalby (n = 254)		Total (n = 1567)	
	n		n		n	
Malocclusion total	886	67 5	155	61 0	1041	66 4
A Anomalies of the dentition	87	6 6	14	5 5	101	6 4
B Occlusal anomalies	840	64 0	142	55 9	982	62 7
(a) Sagittal	618	47 1	90	35 4	708	45 2
(b) Vertical	474	36 1	85	33 5	559	35 7
(c) Transverse	100	7 6	10	3 9	110	7 0
C Deviations of space conditions	89	6 8	22	8 7	111	7 1
D Actual need for treatment	145	11 0	22	8 7	167	10 7
(a) Grinding of teeth	135	10 3	18	7 1	153	9 8
(b) Extraction	1	0 1	0	0	1	0 1
(c) Appliance therapy	2	0 2	2	0 8	4	0 3
(d) Oral surgery	7	0 5	2	0 8	9	0 6

present sucking habits For evaluation of the family s social standard a 3 graded socio-economic grouping system widely used in Sweden was employed This pays special attention to paternal occupation group I representing the highest group (6)

Statistical methods

In the statistical treatment Chi square analyses in case of fourfold tables with Yates correction and tests for normal distribution were used Computations were performed at the Computer Center of Lund University (Univac 1108)

RESULTS

In Lund 1 327 children (90 9 %) and in Dalby 255 children (92 0) appeared at the dental clinic Out of the 154 children who failed to attend 63 children failed the dental examination only while the remaining 91 children did not attend any part of the health control

In Lund the dental examination could be performed on 1 313 children out of 1 327 (98 9 %) 678 boys and 635 girls and in Dalby on 254 children out of 255 (99 6) 129 boys and 125 girls

Malocclusion was a common finding as is shown in Table 1 Most of the deviations were of minor importance but 167 children 16 0 % of children with malocclusion and 10 7 of all children were referred to and treated by an orthodontist

There were slightly more malocclusions among children in Lund than in Dalby but only regarding occlusal anomalies This dif

Table 2 *Connection between malocclusions (open bite frontal overjet postnormal bite crossbite) and sucking habits in 1567 four year old children*

Interview	Dental findings				Total (n = 1567)	
	Malocclusion		No malocclusion			
	n		n		n	% of total
Finger-sucking ended before 2 years of age	19	38 8	30	61 2	49	3 1
Finger sucking ended between 2 and 4 years	12	50 0	12	50 0	24	1 5
Still sucking fingers	352	74 6	120	25 4	472	30 1
Dummy-sucking ended before 2 years of age	132	37 9	216	62 1	348	22 2
Dummy-sucking ended between 2 and 4 years	115	55 0	94	45 0	209	13 3
Dummy still in use	157	93 5	11	6 5	168	10 7
Still sucking both finger and dummy	189	91 3	18	8 7	207	13 2
Total number of children with previous or present sucking habits	751	61 6	469	38 4	1220	77 9
No sucking habits	83	23 9	264	76 1	347	22 1

Form Malocclusion — Form 4

MALOCCLUSION	Dentition		Supernumerary tooth		1
			[illegible]	— —	2
			Aplasia	– –	3
			Non-erupted tooth	— –	4
			Infraposition		5
			Rotated tooth 30° or more		6
			Inverted tooth max 3 teeth	– — –	7
	Occlusion	Sagittal	Maxillary overjet 3-5 mm	– –	8
			Maxillary overjet > 5 mm		9
			Mandibular overjet > 0 mm	– – –	10
			Postnormal bite		11
			Prenormal bite	–	12
		Vertical	Open bite frontal 0-2 mm		13
			Open bite frontal > 2 mm		14
			Open bite lateral > 0 mm		15
			Deep bite frontal > 2 mm		16
		Transversal	Crossbite unilateral		17
			Crossbite bilateral		18
			Scissors bite unilateral		19
			Scissors bite bilateral		20
	Spacing		Maxillary crowding		21
			Mandibular crowding		22
			Maxillary abnormal spacing	–	23
			Mandibular abnormal spacing		24
Supplementary registration			Pronounced general abrasio		25
			Pronounced abrasio single teeth		26
			Sagittal forced bite		27
			Transverse forced bite		28
			Midline displacement ≥ mm		29
			Maxillary medial diastema ≥ mm		30
			Mandibular medial diastema ≥ 2 mm		31
			Abnormal maxillary labial frenulum		32
			Abnormal mandibular labial frenulum		33
			Abnormal lingual frenulum		34
			Pronounced facial asymmetry		35
			Cheilo-gnato-palatoschisis		36
Actual need of treatment			No treatment indicated		37
			No treatment indicated before 7 yrs		38
			Observation		39
			Instruction		40
			Grinding		41
			Extraction		42
			Appliance therapy		43
			Oral surgery		44
			Remarks		45
			No orthodontic annotations		46

Fig 1

Table 4 *Caries and gingivitis related to sucking habits in 1367 four year old children*

Interview	n	Dental findings: Caries Mean	Caries S D	Gingivitis Mean	Gingivitis S D
Finger-sucking ended before 2 years of age	49	5 84	9 21	0 32	0 32
Finger-sucking ended between 2 and 4 years	24	6 46	6 87	0 45	0 41
Still finger sucking	472	5 57	7.24	0 34	0 34
Total	545	5 63	7 41	0 34	0 34
Dummy-sucking ended before 2 years of age	348	10 40	11 03	0 43	0 41
Dummy-sucking ended between 2 and 4 years	209	10 94	10 97	0 45	0 41
Dummy still in use	168	9 97	10 72	0 53	0 43
Total	725	10 46	10 93	0 46	0 42
Using dummy dipped in sugar	48	1 46	13 30	0 45	0 45
Still sucking both fingers and dummy	207	9 51	10 15	0 51	0 47
Total number of children with previous or present sucking habits	1270	8 45	9 98	0 41	0 39
No sucking habits	347	9 11	10 82	0 37	0 37

children with these types of malocclusion were 8 41 ± 10 07 and 0 42 ± 0 40 respectively while corresponding figures for children without malocclusion were 8 81 ± 10 29 and 0 38 ± 0 38 The difference of caries between the two groups was not significant while the difference of gingivitis was significant at the 1 level

The findings of caries and gingivitis in relation to sucking habits are given in Table 4 Children who used a dummy had more caries and gingivitis than children who were or had been finger suckers ($p < 0\,001$) Most caries was found among children who used a dummy dipped in sugar but even if these children were excluded dummy sucking children still had significantly more caries and gingivitis

However sucking habits seem to be socio-economically dependent too the dummy being more popular in lower socio-economic groups and the fingers in higher groups (Table 5) These differences are highly significant $p <$ 0 001 The total sucking habit was equally distributed on the socio economic groups

DISCUSSION

Malocclusion is more seldom considered to warrant correction at this age (25 26) Nevertheless 10 7 of our children were judged to need orthodontic treatment although in the vast majority only *grinding*

Malocclusion is usually caused by hereditary patterns caries or harmful oral habits (2 20) The role of caries as an important etiological factor in misalignment of the mixed or permanent dentition is sometimes questioned (21) while others have found a clear reduction of malocclusion related to water fluoridation and decrease of caries (1 22) In this study of primary dentition the mean caries indices were

Table 5 *Previous and present sucking habits according to socio economic status*

Habits	Socio-economic group: I (n = 477) n	I %	II (n = 558) n	II %	III (n = 537) n	III %	Total (n = 1567) n	Total %
Finger-sucking	41	51 1	198	35 5	106	19 7	545	34 8
Dummy-sucking	136	78 8	239	46 4	330	61.5	725	46 3
Total sucking	362	76 7	435	78 0	423	78.8	1 220	77 9

Table 3 *Single sucking habits and types of malocclusion in 1567 four year old children*

	Number of children with									
	Crossbite		Open bite frontal		Maxillary overjet		Postnormal bite		Any malocclusion	
Habit	*n*		*n*		*n*		*n*		*n*	
Sucking of finger only discontinued ($n=62$)	3	4 8	6	9 7	19	30 6	18	29 0	27	43 5
Sucking of dummy only discontinued ($n=514$)	26	5 0	56	10 9	144	28 0	86	16 7	117	22 8
Sucking of finger only continuing ($n=433$)	30	6 9	142	32 8	189	43 6	123	28 4	320	73 9
Sucking of dummy only continuing ($n=161$)	28	17 4	124	77 0	85	52 8	57	35 4	151	93 8
No sucking habits reported ($n=347$)	5	1 4	6	1 7	66	19 0	35	10 1	83	23 9

ference was statistically significant at the 5% level No sex difference was found in any parts of the malocclusions Among children with malocclusion the mean defs was $8\ 66 \pm 10\ 43$ and the gingivitis index was $0\ 42 \pm 0\ 39$ and in children without malocclusion $8\ 46 \pm 9\ 67$ and $0\ 37 \pm 0\ 38$ respectively The difference of gingivitis indices between these two groups was significant ($p<0\ 05$) but not the difference of caries ($p>0\ 05$)

Table 2 presents the connection between the findings of malocclusions (open bite frontal maxillary overjet postnormal bite and crossbite) and the children's *sucking habits* as reported in the interview of their mothers Previous or present sucking habits were reported for 77 9 of the children 10 7 % still used a dummy 30 1 were sucking their fingers and 13 2% used both dummy and fingers Malocclusion was significantly more frequent among children with earlier or present sucking habits (61 6%) than among children without such habits (23 0%) $p<0\ 001$ Children who used a dummy even if they stopped using it before the age of 2 years, had malocclusion significantly more often than children without any sucking habit ($p<0\ 001$) Malocclusion was also more frequent in finger sucking children than in non sucking children at a significance level of 5 if they stopped before 2 years of age at 1% if they stopped between 2 and 4 years of age and at 0 1% if they were still finger sucking Only 11 out of 168 children (6 5%) still using a dummy had no malocclusion compared with 120 out of 472 (25 4 %) of the still finger sucking children ($p<0\ 001$) To ascertain what specific influence the various sucking habits had on the dental arches, subgroups of malocclusions were related to children with single habits i e children with more than one sucking habit were excluded From Table 3 it is evident that all four subgroups of malocclusion were most frequent among continuing dummy suckers and crossbite open bite and maxillary overjet was second most frequent among continuing finger suckers Postnormal bite had about the same frequency in discontinued as in continuing finger suckers

Considering the differences between the various forms of malocclusions in children with and without sucking habits it is found that discontinued finger-sucking was most frequently connected with postnormal bite and maxillary overjet and discontinued dummy sucking continued finger and dummy sucking with open bite and maxillary overjet in that order All four types of malocclusion were more frequent among both dummy suckers and finger suckers than among non suckers ($p<0\ 001$)

The mean caries and gingivitis indices for

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Key words Pre-school children malocclusion sucking habits

not differentiated in children with and with out malocclusion

Our findings of sucking habits confirm the observation that dummy sucking tends to end earlier than finger sucking (4 7 10 17 18 25 26) The importance of these habits for the development of malocclusion is stressed by several authors (4 5 8 9 19 24 25) Among our children there was a strong statistical con nection between sucking habits and the findings of open bite overjet postnormal bite and crossbite It was also found that dummy sucking, if it continued at the age of 4 years, had a more pronounced effect on the dental arches than finger-sucking The reason could be that the use of a dummy is more frequent and intense during the day than is finger sucking Other studies concluding that dummy sucking is not as harmful to the dentition as finger sucking have dealt with older children (7) or only specific types of malocclusion (9) The facts that many children without sucking habits had malocclusion and that many sucking children still had normal occlusion suggest that other factors probably of hereditary origin play an important role in the development of malocclusion

Unless these sucking habits continue after the age of 6–9 years their consequences to the permanent dentition seem to be of little significance (8 19 23 24 25 26) What seems more serious is that children using dummies had significantly more caries and gingivitis than children using fingers However as they did not have more caries than children without any sucking habits whatsoever factors other than sucking must be involved A further analysis showed that the use of dummies was more frequent in lower socio economic groups, and we already known (15) that these children had poorer oral hygiene (less tooth brushing more frequent between meal eating) Conversely finger-sucking children came from higher socio economic groups and they had better oral hygiene

The conclusion drawn from this study of previous and present sucking habits in 4 year old children as reported by their mothers, is that dummy sucking was more common from infancy although it stopped at an earlier age than did finger sucking Both habits had a significant impact on the development of the deciduous dental arches, most pronounced for continuous dummy sucking The higher prevalence of caries and gingivitis in children using dummies could be referred to other environmental factors Since nowadays there seems to be a common opinion that these types of malocclusion will correct themselves spontaneously if the sucking stops before the permanent dentition is reached, both sucking habits could be considered as rather harmless However only a follow up of the children could provide definite information about the persistence of malocclusion and its relation to sucking habits

In our routine program for preventive dental care within the Child Health Service (15) mal occlusions are also attended to mostly by case finding i e early detection of malocclusion where orthodontic evaluation and treatment is indicated (2) Also in consultation with the paediatrician and the psychologist gentle information and advice on sucking habits are given

SUMMARY

In an unselected population of 4 year old children 66 4 were found to have mal occlusion and 10 7 were considered in need of treatment by an orthodontist Previous or present sucking habits were reported for 77 9% of the children 10 7 still used a dummy 30 1 were sucking on their fingers and 13 2% used both dummy and fingers

A strong statistical connection was found between sucking habits and malocclusion most pronounced for dummy sucking No difference in mean caries index was found between children with and without malocclusion Significantly more caries was demonstrated in dummy sucking children than in finger sucking children but these differences could be explained by other environmental factors

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Fig. 1. Cutaneous rash in Case 1 at 1 year of age.

lytic anemia was not clarified. No signs of autoimmune hemolytic anemia were present. The blood smear showed anisocytosis, poikilocytosis, polychromasia and scattered nucleated red cells. Bone marrow preparations showed very active erythropoiesis, and a number of the erythroblasts contained clover leaf formed nuclei. The hemolytic process was fairly well compensated and he was sent home with the diagnosis hemolytic anemia of unknown cause.

In the spring of 1970 the mother started to take him in the sunshine and soon afterwards he got the typical rash of erythropoietic porphyria on the sun exposed areas with vesicles and blisters which often became infected and on healing left hyperpigmented dots and scars (Fig. 1).

He was readmitted to the hospital in Trondheim August 1970 and this time the diagnosis erythropoietic porphyria was soon established. His teeth showed reddish brown discoloration (Fig. 2) and in Wood's light a strong red fluorescence could be seen. Fresh urine was pink, but after 30 min in daylight the color changed to dark brown.

Porphyrin analyses of urine, feces, plasma and erythrocytes were performed and the results are presented in Table 1. As seen from the table the urinary porphyrins consisted primarily of coproporphyrin and an ether insoluble fraction thought to be uroporphyrin. Feces contained coproporphyrin and smaller amounts of an apparent protoporphyrin. Erythrocytes contained primarily protoporphyrin while copro- and uroporphyrins were not or only moderately increased. Paper chromatography of the urinary porphyrins revealed a mixture of approximately equal amounts of uro- and a 7 carboxylic porphyrin with smaller amounts of 6, 5 and 4 carboxylic porphyrins. Isomer analyses of uroporphyrin and the 7 carboxylic porphyrin could not be undertaken at the time. However, it was found that 80–90% of the coproporphyrin in the patient's urine was the type I isomer.

The patient was placed in a room protected from sunlight. After 2 weeks the hemoglobin levels continuously rose from 8.5 to 11.3 g/100 ml and reticulocytes decreased from 6.1 to 1.8%. The skin lesions healed and new blisters did not occur. It was found practical and effective to cover the window panes with curtains of a yellow celluloid film ("Para Sol") which has a good translucency for light with wavelengths down to 550 nm but is not translucent for light with wavelengths below 510 nm. Such curtains have consequently been used in his home with good results in prevention of skin rash. The patient has not been allowed to go out in daylight without protection of all parts of the body. On the head he has used a cowboy hat with yellow celluloid film hanging down as a shelter for his face and neck.

In the autumn of 1971 protection of the sun exposed areas with an ointment ("Mexenone") (10) was tried. However, after some weeks the condition deteriorated and the former careful light protection had to be reinstituted. He was admitted to the Department of Paediatrics, University Hospital, for further studies April 13 to June 16, 1972. He was in good general health without skin rash. The spleen was palpable 1 finger below the costal margin, moderately enlarged. The urine was pink, and contained 11 600 μg porphyrin per day. Paper chromatography showed that the excreted porphyrins were a mixture of 4 330 μg uroporphyrin, 4 330 μg 7 carboxylic porphyrin, 775 μg 6 carboxylic, 860 μg 5 carboxylic, 1 270 μg coproporphyrin and a small amount of a porphyrin behaving as a 3 carboxylic porphyrin. Feces contained large amounts of coproporphyrin, but considerable amounts of a porphyrin were found having solubility properties and behaving chromatographically as a dicarboxylic porphyrin. The hemoglobin level on admission was in the range of 7.5–8.0 g/100 ml and remained at this level for weeks. Bone marrow smears showed a marked erythropoietic hyperactivity and a number of the erythroblasts had abnormal nuclei with inclusions and/or clover leaf

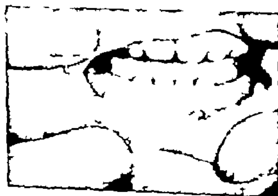


Fig. 2. Discolored teeth in Case 1.

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CONGENITAL ERYTHROPOIETIC PORPHYRIA WITH A HITHERTO UNDESCRIBED PORPHYRIN PATTERN

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Congenital erythropoietic porphyria (CEP) or Gunther s disease is a rare inborn disorder of heme synthesis, which has not been described hitherto in Scandinavia However two siblings born in the USA from Norwegian descended parents have been reported (1 personal communication) indicating that the gene may be present in the Norwegian population In a recent review by Marver & Schmid (11) 60 cases reported up to 1963 were summarized They state that at least 10 more cases have been described since 1963 The purpose of this report is to present clinical findings and laboratory data of a patient from Northern Norway suffering from CEP and of his second cousin who possibly had the same disease In later communications more detailed studies of porphyrin metabolism in the patient and his family will be presented

METHODS

Urine was collected over 24 hour periods and ether soluble and ether insoluble porphyrins separated and quantitated essentially according to Rimington (16)

Fecal porphyrins were extracted and quantitated according to Rimington (16)

Erythrocyte and plasma porphyrins were extracted as previously described (6) however the cyclohexanone technique of Rimington (16) was introduced for the purification and quantitation of the ether insoluble porphyrins

Paper chromatography of porphyrin free acids was performed according to Eriksen (5) The porphyrin zones were cut out with a scissor eluted with 3 N HCl and quantitated Porphyrins having 7 6 and carboxylic groups were determined as thou h th w re uroporphyrins while 3 carboxylic porphyri were quantitated as though they were coproporphyrin 8 and 7 carboxylic porphyrins were esterified w methanol/H_2SO_4 (95/5 v/v) for 16 hours the meth esters brought into chloroform and precipitated w petroleum ether The precipitated porphyrin este were decarboxylated to free coproporphyrins accor ing to Edmondson & Schwartz (4) and purified chr matographically as mentioned above The isom composition of the different coproporphyrin fractio obtained was determined according to Eriksen (7)

Otherwise routine hematological methods were us in the study of the patients

CASE REPORTS

Case 1

This boy M W was born at term on July 6 196 following normal pregnancy and delivery as No 4 four siblings Birth weight 2 680 g, length 46 cm H two older sisters are healthy while a brother died b accident when 2 years old The parents are health and not consanguineous A second cousin of th patient (Case 2) died in infancy with a similar disea picture

From the very first day of life the mother ob erve that the boy s urine often colored the diapers re This observation however was not mentioned to th hospital staff until nearly 1 year later and an im portant clue to the correct diagnosis was therefor overlooked From 5 weeks of age the patient showe failure to thrive He was found to be anemic (H 6 5 g 100 ml) and was admitted to Trondheim syke hus Trondheim 7 weeks old The spleen was palpabl 3 cm below the costal margin A hemolytic anemi with 6 2–7 5% reticulocytes was disclosed 4 week later he was transferred to the Department o Pediatrics University Hospital Oslo

During these hospital stays the cause of the hemo

transfusions have been reported (1 19) Even early death from severe anemia has been de scribed (17 18) Unstained bone marrow smears show intense fluorescence in nucleated red blood cells in the fluorescence microscope Many fluorescing normoblasts may in their nuclei contain 1 or more inclusions which stain dark with benzidine Patient M W (Case 1) shows all the characteristic clinical features of CEP Whether his second cousin (K E W Case 2) also had CEP is probable but not certain He died however less than 10 months old with fever and falling hemoglobin levels before porphyrin studies were performed In contrast to the first patient he had moderate thrombocytopenia This might be explained by the relatively marked splenomegalia Thrombo cytopenia has been observed in other patients with CEP (18)

Patients with CEP excrete large amounts of uroporphyrin in the urine The urinary excre tion of coproporphyrin is also markedly in creased while porphyrins having 7 6 or 5 carboxylic groups are only moderately in creased (2) Most of the urinary porphyrins are of type I isomer Large amounts of copro porphyrin I are demonstrable in the feces the fecal content of uroporphyrin is usually small Circulating red cells contain high concentra tions of uroporphyrin I somewhat lower con centrations of coproporphyrin I and variable concentrations of uroporphyrin I and copro porphyrin I are found in the plasma

In our patient (Case 1) a different porphyrin pattern has been found Both 8 7 6 5 and 4 carboxylic porphyrins are considerably in creased in the urine However the most im pressive finding is the extraordinary large amounts of 7 carboxylic porphyrin which re presents at least the same amount as uro porphyrin The same porphyrin pattern has been found in all samples so far studied by us (at least 30 samples over a period of approxi mately 2 years) and independently by Rimington & With (personal communication) in a sample from June 70 Isomer analysis has shown this porphyrin to belong to th isomer III series This urinary pattern is more like that found in porphyria cutanea tarda (9) than that of any of the known erythropoietic por phyrias The pattern found in the blood also varies considerably from that said to charac terize CEP In the erythrocytes protoporphyrin dominates quantitatively and porphyrins hav ing four or more carboxyl groups are not or only moderately increased and plasma con tains not only moderate amounts of both uro- and coproporphyrin but also considerable amounts of protoporphyrin The blood pattern is more like that found in erythropoietic proto- porphyria than in CEP The significance of these and other findings will be discussed in a separate article The typical clinical picture the enormous increase of uroporphyrin in the urine and the presence in the bone marrow of erythroblasts with fluorescence in the nuclei definitely establish the diagnosis CEP The findings of large amounts of 7 carboxylic por phyrin in the urine and increased amounts of protoporphyrin in feces and plasma are dif ferent from what is found in classical CEP and indicate that CEP may be genetically heterogeneous

Treatment consists first of all of protection against daylight Some patients may tolerate ordinary daylight when it is overcast others need more strict light protection even of their indoor environment Ointments do not seem to be effective enough We have found a yel low celluloid film (Para Sol) useful and have used this with good results to cover the window panes in M W s bedroom and also to protect his face and neck when he is outdoors Splenectomy was first tried and observed to improve the photosensitivity in the case re ported by De Marval & Pons (3) Aldrich et al (1) reported marked improvement also of the hematological and biochemical findings fol lowing splenectomy in a case of Norwegian descent Lately a number of patients with CEP have been splenectomized with subsequent amelioration of the disease picture but the operation is not helpful in all cases (11) Splen ectomy should be tried in the more severe

Table 1 *Porphyrins in urine feces red cells and plasma in Case 1 August 1970*

	Uroporphyrin	Coproporphyrin	Protoporphyrin	ALA (chromato graphic method)	PBG
Urine	20 8 mg/g creatinine (normal up to 0 015 mg)	3 8 mg/g creatinine (normal up to 0 1 mg)	—	0 19 mg/100 ml	Not demon strable
Feces	Not demonstrable	320 μg/g dry feces (normal up to 20 μg/g)	150 μg/g dry feces	—	—
Red cells	Not demonstrable	7 0 μg/100 ml (normal up to 1 0 μg/100 ml)	474 μg/100 ml (normal up to 50 μg/100 ml)	—	—
Plasma	37 μg/100 ml	21 μg/100 ml	7 9 μg/100 ml	—	—

form Fluorescence microscopy of unstained smears showed large numbers of fluoroblasts The fluorescence was primarily located in or on the nuclei and the intensity of the fluorescence increased with increasing maturity of the erythroblasts

Plasma contained 63 0 μg uro 62 2 μg copro and 48 4 μg protoporphyrin/100 ml and the erythrocytes 480 4 μg protoporphyrin trace amounts of copro porphyrin while uroporphyrin was not demonstrable

All these findings are essentially the same as those made in 1970

Case 2

K E W was a boy also from Northern Norway born March 3 1969 following normal pregnancy and delivery as No 6 of six siblings He was a second cousin to Case 1 their fathers being first cousins Information about the patient s health during the first months of life is incomplete At 6 months of age he was admitted to the local hospital with severe hemolytic anemia failure to thrive and periods of fever Sept 24 1969 he was transferred to the Department of Paediatrics University Hospital Oslo where he stayed until Dec 13 1969 His hemolytic anemia was similar to that in Case 1 although more severe On admission hemoglobin was 4 2 g/100 ml and during the hospital stay six blood transfusions had to be given The spleen was somewhat more enlarged than in Case 1 and in contrast to his cousin he had a reduced platelet count usually about 50 000 (range 16 000–100 000) There were no signs of auto immune hemolytic anemia congenital spherocytosis pyruvate kinase or G 6 P D deficiency (other red cell enzymes were unfortunately not studied) Blood and bone marrow smears were very similar to those of Case 1 The urine on several occasions gave a red staining of the diapers This patient also had a diffusely enlarged heart without signs of a congenital cardiac malformation (caused by the anemia ?)

In Nov–Dec 1969 his hemoglobin level remained stable between 9 and 10 g/100 ml for more than 3 weeks without blood transfusions and he was tentatively sent home Less than 2 weeks later he got fever and increasing anemia and he died on his way to hospital Dec 26 1969

COMMENTS

The first clinical signs suggesting the diagnosis CEP is usually the excretion of red urine due to the presence of uroporphyrin in high amounts This is most often noted in early infancy sometimes even at birth The red color of the urine may show considerably daily and seasonal fluctuations with exacerbations following exposure to sunlight The photosensitivity and typical vesicular or bullous rash are usually discovered around 1 year of age as in Case 1 Marked scarring and mutilation may ensue with contractions of the face and loss of digits and ears In other patients the eruptions tend to be milder leaving only moderate sequelae

Hypertrichosis is common with fine blond downy hair The teeth show a red or brownish discoloration and marked fluorescence in ultraviolet light

Splenomegaly of varying degree is found in the great majority of the patients

The patients show increased hemolysis In most patients reported the hemolytic anemia has been relatively well compensated with only slight reduction of hemoglobin concentration But the anemia may be quite severe as in our two cases Patients requiring repeated blood

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CONGENITAL ERYTHROPOIETIC PORPHYRIA

The Effect of Light Shielding

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A case of congenital erythropoietic porphyria (CEP) with a hitherto undescribed porphyrin pattern has recently been described (5) In the present paper evidence will be presented indicating that the decreased stability of circulating red cells in CEP is directly related to photocatalytically induced changes of the red cells It will also be shown that the overwhelming part of free porphyrins found in the urine is formed photochemically from excess porphyrinogens when the skin and blood are exposed to wavelengths below 510 nm The possible significance of these and other findings will be discussed

MATERIALS AND METHODS

The patient (M W) a boy born at term on July 8 1969 has been described previously (5)

He has been under continuous study since August 1970 and repeated measurements of urinary and blood porphyrins and the hemoglobin levels have been performed The techniques used for the extraction separation and quantitation of porphyrins have been described in previous papers (5) However it was early learnt that part of the porphyrins found in the urine was formed from porphyrinogen either due to light exposure of the urine or to the extraction procedures used To get a measure of the amounts of porphyrinogens present in different samples the following procedures were developed The urine was acidified with glacial acetic acid to pH 4 and extracted twice with two volumes of ether/glacial acetic acid (5 1) washed once with water extracted with 0 1 N HCl until no more red fluorescing material could be brought out of the ether Unoxidized porphyrinogen in the ether phase was converted to porphyrin and brought out of the ether either by the I procedure of Rimington (11) or by 3 N HCl extraction as previously described (2) The ether soluble porphyrins brought out with 0 1 N HCl were re extracted with ether/glacial acetic acid (5 1) after addition of crystalline sodium acetate to pH 4 It was found that most of the previously ether soluble porphyrins" had become ether insoluble during the extraction procedure and direct measurements in the spectrophotometer showed that the maximum extinction in the Soret band of the 0 1 N HCl extracted pigments could increase 50% or more when the extracts were left on the laboratory bench in the light for an hour or two Apparently varying amounts of porphyrins were formed during the extraction procedure

The ether insoluble porphyrins formed during the first HCl extraction procedure were extracted with cyclohexanone at pH 3 5 and brought out again without measurable increase after addition of 2 volumes ether and extraction with 3 N HCl Paper chromatography (3) of these porphyrins revealed that they were composed of primarily porphyrins having 7 carboxylic groups and minor amounts of 6 and 5 carboxylic porphyrins The ether-soluble porphyrins brought into ether by re extraction were readily brought out with 0 1 N HCl and no or only negligible additional porphyrin was formed by the I procedure or by light exposure On paper chromatography only coproporphyrin and minor amounts of porphyrins having 5 and 6 carboxylic groups were found

The primary ether insoluble porphyrins in the urine and wash water were extracted with cyclohexanone in the usual way and preformed porphyrin and porphyrinogen determined according to Rimington (11) Paper chromatography revealed primarily uroporphyrin and 7 carboxylic porphyrins with minor amounts of 6 5 and 4 carboxylic porphyrins The total amount of porphyrinogen was estimated as the sum of I (or 3 N HCl) formed porphyrins plus the

cases The recent finding that vitamin E may play an important role in the regulation of porphyrin biosynthesis (13 14) and that vitamin E has a beneficial effect upon the photosensitivity in porphyria cutanea tarda (15) indicates that vitamin E might have a beneficial effect also in the present disease β-carotene has been shown to have a beneficial effect on the photosensitivity in erythropoietic protoporphyria (12) thus also this compound is worth a trial

SUMMARY

One proven and one probable case of CEP in second cousins from Northern Norway are reported The porphyrin pattern in Case 1 who has been studied extensively in certain respects differs from that reported earlier in CEP indicating that the disease may be genetically heterogeneous The most impressive finding is the extraordinary high amounts of 7 carboxylic porphyrin in urine Isomer analysis has shown this porphyrin to belong to the isomer III series The presence of increased amounts of protoporphyrin in plasma and feces is also at variance with the picture seen in classical CEP

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skin manifestations except when the patient occasionally slipped outdoors for an hour or two On these occasions the urine always was red when voided and a rash constantly appeared on light exposed areas on the next day

A change in the treatment from light shielding with filters to the ointment Mexenone (7) lead to a drop in the hemoglobin level from 90 to 75 At the same time the erythrocyte porphyrin picture changed back to predominantly protoporphyrin minor amounts of copro and not detectable uroporphyrin The latter picture has been found to be constant in all periods with anemia The spleen was also followed by repeated examinations and as seen from the table splenomegaly was apparent in all phases of anemia with excretion of large amounts of free porphyrins The spleen continued to grow even when no uroporphyrin was detectable in the red cells

The amount of urinary porphyrins increased during the whole period of observation and seemed to be unaffected by the treatment The ratio free porphyrins/porphyrinogens in the urine changed however markedly with the efficiency of the light shielding During the period of effective light shielding porphyrinogen increased on the cost of free porphyrin and *vice versa* when light shielding became ineffective

DISCUSSION

An important finding in the present case of CEP is the overproduction of porphyrinogens To what extent the excess of formed porphyrinogens are oxidized to porphyrins *in vivo* is difficult to say at the present However the above findings show that a very considerable part of excessively formed porphyrinogen will be excreted as such if photochemical conversion in the skin is avoided by effective light filtering The excretion of primarily porphyrinogens in the urine in human CEP has to our knowledge never been described before It is however a usual feature in bovine CEP at least in the winter time (6 8) In the summer time when the cattle is directly exposed to sunlight the color of the urine may change from the typical greenish to reddish brown and the urine shows intense red fluorescence in Wood's light (With personal communication)

We have previously (5) shown that the urinary porphyrin pattern in the present case is more like that seen in porphyria cutanea tarda (CT) than that said to characterize CEP These findings and the present findings that effective light shielding causes the urinary pattern to change from predominantly free porphyrins to predominantly porphyrinogens are similar to those made by Burnett & Pathak (1) in two cases of CT and indicate that the inborn error of porphyrin metabolism is the same in the present case of CEP as in CT and that the only difference is the tissue location

The finding that the patient was highly photosensitive during the whole period of observation even in periods in which the only porphyrin increased above normal in the erythrocytes was protoporphyrin seems to indicate that the skin rash is primarily due to the presence of porphyrins/porphyrinogens in plasma Our previous finding that feces contains a dicarboxylic porphyrin apparently protoporphyrin (5) and the present finding that protoporphyrin is found in plasma in all phases of the disease in certain respects connect the present case with erythropoietic protoporphyria (EPP) in which photosensitivity of the skin seems to be directly related to the presence of protoporphyrin in plasma (9) It is not possible at present to decide whether the skin rash appearing when the skin is exposed to unfiltered daylight is due to the chemical processes which start when porphyrinogens are converted to porphyrins on appropriate light stimulation as suggested by Burnett & Pathak (1) in CT or due to the photocatalytic activity of free porphyrins formed by light exposure or oxidation by the cell itself The fact that all samples of plasma studied showed primary red fluorescence in Wood's light in all phases of the disease demonstrates that at least some of

Table 1 *The effect of light shielding on the amounts of erythrocyte plasma and urinary porphyrins degree of anemia splenomegaly and photosensitivity in a case of CEP followed from Aug 1970 to April 1972*

Type of treatment	Effective light shielding with Para-Sol filters				Ineffectively treated with Mevenone	Varying types of incomplete light shielding	
Time	Aug 70	Jan 71	May 71	June 71	Oct /Nov 71	Jan 72	Apr 72
Erythrocytes µg/100 ml							
Proto	474 2	726 1	576 7	+	567 9	+	364 4
Copro	7 0	73 5	26 2	+	trace	+	trace
Uro	–	+ +	+	+	–	+	–
Plasma µg/100 ml							
Proto	7 9	22 4	20 1	+	62 1	+	48 4
Copro	21 1	62 0	40 1		11 5		62 3
Uro	37 2	48 9	23 1		13 6		63 2
Fluorescence	red	red	red		red		red
Urine µg/day							
Total	3 100			6 500	7 800	9 700	13 000
Porphyrin/Porphyrinogen ratio	90/10			20/80	40/60	90/10	90/10
Colour	red	yellow	yellow	yellow	yellowish/brown	red	red
Condition							
Hb	50	90	90	90	75	55	50
Splenomegaly	+ + + (+)	–	–	–	+	+ + +	+ + + (+)
Photosensitivity	The patient was highly photosensitive during the whole period						

difference between the amount of ether-extractable porphyrins in the urine and the amount of ether extractable porphyrins found on re-extraction of the first fraction

As judged from the color and intensity of the fluorescence of the urine and the final extracts these procedures give a quite good picture of the amount of porphyrinogens and preformed porphyrins present in the urine when studied Since the urine was collected over 24 hour periods and oxidation of porphyrinogens most probably begins already in the bladder these figures do not represent the true excretion pattern

The ratio preformed porphyrin/porphyrinogen in erythrocytes was not possible to quantitate due to the quenching effect of the enormous amount of heme present

Plasma was also difficult to quantitate however the lack of other disturbing pigments allowed direct inspection in Wood s light and it was possible to decide whether preformed porphyrins were present or not in the plasma samples studied

RESULTS

The results of the present investigations have been collected in Table 1 As seen from the table several parameters vary considerably with the type of treatment used

Shielding against light of wavelengths below 510 nm lead to disappearance of the anemia and a marked change in the erythrocyte porphyrin pattern In August 1970 when the patient was heavily diseased with skin rash and marked anemia only protoporphyrin was significantly increased in the erythrocytes copro porphyrin was only moderately increased while uroporphyrin was not detectable In January and May 1971 when the patient was effectively shielded against the shorter wave lengths of daylight the anemia kept moderate and well compensated, the erythrocytes contained increased amounts of both proto copro and uroporphyrin and according to the mother the urine was normal in color when voided but became reddish brown to bluish black when the diapers were left for some time The general health was good with no

constant An increased destruction of faulty red cells in the spleen is also indicated by the finding that the spleen continues to grow even in periods when the blood contains apparently normal erythrocytes if the light shielding is ineffective (Table 1 Nov 71 – Apr 72)

The finding of Pfeiffer & Tatum (10) that the spleen will clear the blood for trypanosomes injected intravenously to a concentration of 1/20 of the red cells in less than 15 min when the trypanosomes are made "foreign by injection of the appropriate dose of Mafarsen shows that the capacity of the spleen to remove foreign particles far exceeds that needed to remove faulty red cells in CEP

If as the findings in the present and previous papers (5) indicate CEP is genetically heterogeneous and the destructive effects which the inborn error in porphyrin biosynthesis causes in this disease are due not to the inborn error *itself* but to its tissue location and to light induced photochemical processes in the blood and skin it would be easier to explain why the gene has been able to spread so effectively as it has in cattle and why a species like the squirrel *Sciurus niger* having a similar metabolic defect in porphyrin biosynthesis in homozygous dose (14) shows no clinical signs of disease

In bovine CEP the skin reactions seen in summer time are usually found in areas with white hair (With personal communication) Apparently the colored hair represents an effective light filter in this species The black fur of *Sciurus niger* effectively protects the animal against the disastrous effects of daylight and represents a very strong positive selection factor which most probably is the reason why this species has been able to survive in spite of its severe metabolic disorder

SUMMARY

The effect of various types of measures taken for light shielding against daylight in a recently detected case of CEP has been followed for more than a year It has been found that the celluloid film Para Sol which has a good translucency for wavelengths above 550 nm but is not translucent to wavelengths below 510 nm not only removes the troublesome skin lesions seen in this disease but also brings about an almost complete compensation of the anemia disappearance of the splenomegaly and marked changes in the blood and urinary porphyrin patterns In blood the erythrocyte porphyrins change from predominantly protoporphyrin with trace amounts of copro- and not detectable uroporphyrin to high amounts of both proto- copro and uroporphyrins The plasma porphyrins remain essentially unchanged The urinary pattern changes from predominantly free porphyrins to predominantly porphyrinogens

It is suggested that the present case of congenital erythropoietic porphyria represents a new type of CEP and that the hemolytic anemia with splenomegaly seen in CEP is due not to the inborn error itself but to its tissue location and photochemical effect of light of wavelengths below 510 nm when the porphyrin/porphyrinogen rich erythrocytes are exposed to daylight in the skin while the skin lesions have been shown to depend primarily on the presence of an increase in the amounts of porphyrin/porphyrinogen in plasma Cattle and the species *Sciurus niger* are mentioned as examples of species in which the light-shielding effect of the fur has allowed the CEP gene to spread

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the plasma porphyrins must be present as such not as porphyrinogens Free porphyrins are seen in teeth and in the erythroblasts of the marrow This makes it clear that definite con clusions can not be drawn at the present

Whether porphyrin-peptide conjugates so-called X porphyrins (12) play a role in the present case is uncertain Preliminary studies by Rimington (personal communication) in limited samples of feces and urine from the present case have given no indication that X porphyrins are present in the excreta A closer study is however needed before definite con clusions can be drawn

Our finding that effective light shielding not only prevents the skin rash to appear but also causes the splenomegaly and anemia to disappear suggest that both the anemia and the splenomegaly are due to photocatalytically induced changes of the circulating erythrocytes leading to foreignness of these cells with subsequent phagocytosis in the spleen and growth of the latter

If this turns out to be correct, it would be easier to understand why chronic light ex posure leads to splenomegaly and anemia in CEP while in CT neither anemia nor spleno-megaly are found In CEP the excess por phyrinogens would accumulate inside the developing erythrocytes, and on exposure to daylight in the skin vessels, porphyrins would be formed, not only in plasma and tissue fluids but also within the red cells The photo catalytic process itself or the porphyrins formed, would in CEP lead to foreignness of the red cells with increased destruction in the spleen

In CT excess porphyrinogen formation takes place in the liver and the porphyrinogens formed are excreted into plasma and converted photocatalytically to porphyrins (1) when ex posed to light in the skin The porphyrins formed would be expected to bind to plasma proteins, and thus become unable to penetrate the red cell membrane

The finding in the present case that the anemia continues to develop even when the erythrocytes contain no measurable amounts of uroporphyrin seems to indicate that this porphyrin plays no role in the mechanisms causing the red cells to become foreign when exposed to light However as shown by Watson et al (15) the uroporphyrin rich erythrocytes both in human and in bovine CEP are much more fragile than erythrocytes containing little or no uroporphyrin The presence of a minor group of short lived erythrocytes in human CEP has also been described by Heilmeyer et al (4)

The most reasonable explanation of these findings seems to be that uroporphyrin/uro-porphyrinogen containing erythrocytes when exposed to light of the appropriate wave lengths are maid foreign and removed so rapidly from the circulation by the spleen that the concentration of these cells will be too low to allow the detection or so low that only moderate increases are found It is worthwhile to mention in this connection that Stich (13) found in a case of CEP in which the daily excretion of uroporphyrin often exceeded 50 000 μg that the amount of fluorocytes in the peripheral blood was only 4% and that it is a common feature in CEP that the content of uro and coproporphyrin in the circulating erythrocytes is only moderately increased (8) These and other findings have been used as evidence for the assumption that the over whelming part of porphyrin found in the ex creta in CEP origins from excess porphyrins released from the erythroid cells in the bone marrow or from local destruction in the mar row of defective porphyrin/porphyrinogen rich erythroblasts and erythrocytes As is ap parent from the above findings a considerably larger part than hitherto suspected may origin from destruction of uroporphyrin rich red cells in the spleen This assumption is sup ported by the finding that effective light shield ing not only causes the anemia to disappear but also leads to an increase in the concentra tion of uro and coproporphyrin in the erythro cytes to levels significantly above normal, while the plasma porphyrins seem to remain almost

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QUINIDINE TOXICITY IN A NORMAL HEART

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This report describes the ECG changes induced in a healthy child who ate an unknown number of 200 mg quinidine sulphate tablets

Quinidine is well-documented as a cause of cardiac arrhythmia syncope or sudden death in individuals with heart disease (18) but little is known about its effects on the healthy heart Spera (16) illustrates aberrant ventricular conduction in a previously well 5 year old boy who died after eating 1 5 g of quinidine Kerr et al (7) describe ECG changes occurring after the ingestion of 4 g by a healthy adult who survived

CASE REPORT

Paul C aged 16 months (weight 12.25 kg) ate a large meal at 16 15 At 17 45 he was found chewing a 200 mg tablet of quinidine sulphate It was uncertain whether further tablets which belonged to a relative were missing He was given 8 ml of syrup of ipecacuanha in the Casualty Department and vomited at 19 00 and several times thereafter The vomitus appeared somewhat granular On examination he was a crying healthy child with a heart rate of 130/minute At no time did he become shocked hypotensive or ill No further treatment was administered until 24 00 at which time because of ECG changes he was digitalised (0.5 mg digoxin i.m 8 hourly for 3 doses) and was also given 10 mg frusemide i m The next morning he was perfectly well and at follow up 1 month later he had a clinically normal heart chest X ray and ECG were also normal

ELECTROCARDIOGRAPHIC CHANGES

The first recording (Fig 1) at 21 15 on 5 8 70 3 1/ hours after ingestion of quinidine shows a sinus tachycardia with a rate of approximately 158/minute and a normal QRS complex Fusion of P and T waves makes the exact measurement of the Q T interval impossible but it is not less than 0 24 sec

By 22 15 (Fig 2) the trace is much more abnormal the rate is faster (approximately 170/minute) and the QRS complexes show complete left bundle branch block No P waves are identifiable the rhythm is regular At this stage it appeared that these complexes reflected either supra ventricular beats conducted with left bundle branch aberration or a ventricular tachycardia

At 22 45 (Fig 3) 5 hours after ingestion the child had an episode of retching after which the trace altered abruptly and now shows a sinus tachycardia with a 2 1 atrioventricular response narrower QRS complexes and a rate of approximately 93/minute the conducted beats have a prolonged P R interval (0 2 sec)

At 23 30 the pattern reverted to left bundle branch block and was identical with Fig 2 Over the next 2 hours the trace fluctuated between the appearances in Figs 2 and 3 A change to 2 1 atrio ventricular response was associated on at least two occasions with



Fig 1 3 1/ hours post ingestion of quinidine sinus tachycardia. Paper speed 25 mm/sec in all tracings

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Fig 4 Complex arrhythmia with runs of tachycardia frequent ventricular extrasystoles intermingled with sinus beats and aberrantly conducted beats having a RS pattern

recording was apparently normal except for the prolonged Q T interval of 0 46 the T wave being deformed by a U wave

On 7 8 70 40 hours after ingestion (Fig 5) the Q T interval is 0 28 and the cycle length of the trace suggests a Wenckebach Type of sino atrial block possibly due to mild digitalis intoxication

DISCUSSION

These electro-cardiographs demonstrate the rather striking changes that occurred in the electrical activity of a healthy human heart exposed to quinidine The main changes were A V block and probably ventricular tachycardia They developed without producing any clinical effects other than tachycardia but it is impossible to know how close our patient came to developing ventricular fibrillation or asystole

Acute quinidine toxicity

The acute toxicity of quinidine on the normal heart has been worked out using dogs given large doses (30–60 mg/kg) of the drug (10

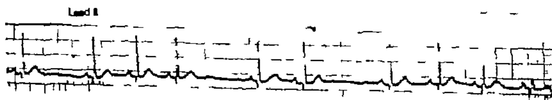


Fig 5 40 hours post ingestion Q-T interval now 0 28 possible Wenckebach Type of sino atrio block is present

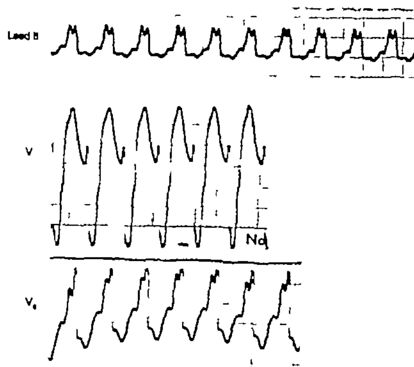


Fig 2 4½ hours post ingestion rate 170 per minute QRS complexes show pattern of complete left bundle branch block no identifiable P waves present Interpretation supraventricular or ventricular tachycardia

struggling or retching In these later tracings there is no evidence that the left bundle branch block complexes are conducted beats and we conclude that they represent attacks of ventricular tachycardia

In the ECG at 00 15 on 6 8 70 6 5 hours after ingestion the Q-T interval is prolonged at 0 4 sec At 01 00 a further physical disturbance was associated with an alteration to a more complex arrhythmia (Fig 4) Runs of tachycardia are seen in this tracing which if considered to be proceded by P waves could be supraventricular in origin, or if considered to be preceded by large U waves deforming the S-T segments could be ventricular There are also frequent ventricular extra systoles often occurring in pairs intermingled with sinus beats and aberrantly conducted beats having an RS pattern

By 08 15 14 5 hours after ingestion the

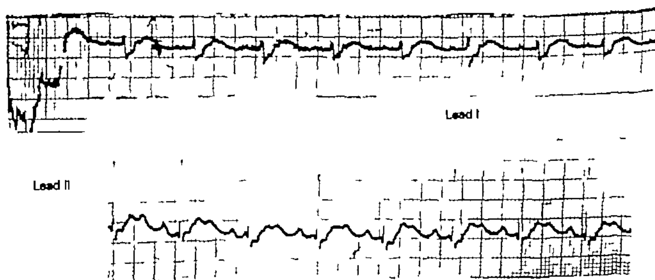


Fig 3 5 hours post ingestion retching episode followed by abrupt change to 2 1 atrio ventricular response the rate is 93 per minute Interpretation probable quinidine toxic effect on atrio ventricular node

tricular fibrillation and possibly respiratory arrest will almost certainly develop In these extreme circumstances cardio pulmonary bypass should be available for an attempt to tide the patient over the few hours when he would otherwise certainly die

SUMMARY

ECG changes are documented in a child who survived ingestion of an unknown quantity of quinidine sulphate Atrial tachycardia developed with intermittent 2 1 A V response and recurrent attacks of probable ventricular tachycardia occurred A plan for the management of acute quinidine poisoning is suggested

ACKNOWLEDGEMENTS

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14) Because of a direct action on the heart, ventricular contraction becomes weaker but the rate may increase due to vagal block As there is also a peripheral vasodilation hypotension may be profound The rate of conduction is slowed the refractory period increased and ectopic foci suppressed The Q-T interval lengthens With doses at the upper end of this range sino atrial arrest may occur and atrioventricular junctional rhythm may supervene which usually progresses to ventricular fibrillation or occasionally asystole

Deliberate studies on healthy humans are few and incomplete but indicate a similar picture (3 17)

It is worth noting that the cat (5) and guinea pig (8) unlike the dog, may die from respiratory arrest while the heart is still contracting effectively Central nervous system effects with coma and convulsions were prominent in Kerr's case (7)

Changes in the ECG may begin within 15 minutes of an oral dose and maximum blood levels are reached between one and fours hours after ingestion (2, 12) Blood levels fall to 40% of the peak level by 12 hours and to 10 by 24 hours after a single oral dose (2, 6) For equal dosage the plasma concentration of quinidine has been shown to vary widely Sokolov (15) noted that signs of toxicity were infrequent at plasma levels of less than 6–8 mg/l

Treatment

At present there is no direct pharmacological antagonist to quinidine available and attempts to reverse acute poisoning in dogs have led to rather conflicting reports (1 9 10 19)

There is no coherent study on the use of either digoxin or a diuretic as used in our patient Neither Luchi et al (10) nor Sierra et al (14) were able to confirm earlier favourable reports of the use of sodium lactate infused intravenously in reversing the effects of a large dose of quinidine The former group demonstrated that angiotensin infused at 3 μg/minute did reverse the hypotension this drug being more effective if calcium versenate was also given Sierra et al (14) noted reversal of ECG changes following administration of 15–30 ml/kg of 0 3 M solution of THAM infused over about 30 minutes in seven of nine dogs in whom the changes had been previously thought to be irreversible, but THAM had little effect on the hypotension

From these results, with which we were not familar at the time of treating our patient, we would suggest the following treatment rather than that which we actually used The stomach should be emptied and ECG and blood pressure recordings begun In the event of hypotension an angiotensin and calcium versenate infusion should be started, the rate to be titrated against the BP response (dose angiotensin amide 0 01–0 2 μg/kg min together with calcium disodium versenate 5 mg/kg min, the latter not to exceed a total dose of 50 mg/kg) Although there is no firm theoretical basis for its use it would seem reasonable to try THAM if an atrioventricular junctional rhythm or a frank ventricular arrhythmia develops (dose 15 ml 0 3 M THAM/kg over 1 hour maximum 300 ml)

In practice differentiation between supraventricular tachycardia with conduction delay and ventricular tachycardia may be difficult as in the present case Isolated successes have been claimed for treatment of ventricular fibrillation with propranolol (13) or external cardiac d c shock (11) in patients taking the drug for heart disease and these approaches to this complication may be worth considering In Kerr's case the use of a demand pacemaker was rendered ineffective by a very high electrical threshold (7)

Excretion of the drug occurs faster with an acid urine the average urine concentration of quinidine being 115±48 mg/l at urine pH less than 6 and 13±8 mg/l at pH more than 7 5 in subjects on a constant daily dosage (4) However acidification of the urine would be incompatible with THAM therapy

If a really large dose of quinidine has been ingested it would seem that irreversible ven

tricular fibrillation and possibly respiratory arrest will almost certainly develop In these extreme circumstances cardio pulmonary bypass should be available for an attempt to tide the patient over the few hours when he would otherwise certainly die

SUMMARY

ECG changes are documented in a child who survived ingestion of an unknown quantity of quinidine sulphate Atrial tachycardia developed with intermittent 2 1 A V response and recurrent attacks of probable ventricular tachycardia occurred A plan for the management of acute quinidine poisoning is suggested

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COMBINED SUPRAPUBIC ASPIRATION AND CLEAN VOIDED URINE EXAMINATION IN INFANTS AND CHILDREN

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The difficulty of distinguishing true urinary infection from contamination of urine samples has in recent years been the interest of several investigations (2, 4 5 6 9 12 13 14 16 19 22, 24) In the pediatric age group this problem is most often encountered in infants between 0 and 2 years at which age urine is routinely collected as bag specimens Several authors (1 3 4 5 18 20 22, 23) have reported that bacterial contamination can be avoided by suprapubic bladder aspiration whereas contamination as judged by leucocyturia has not been systematically studied

The aim of this investigation has been (i) to make a comparison between suprapubic aspirated urine and concomitantly obtained clean voided urine with regard to the bacterial and leucocyte counts, respectively and (ii) to evaluate—through this combined procedure—leucocyte counting as a criterion of true urinary infection

MATERIAL AND METHODS

Percutaneous suprapubic aspiration of bladder urine was performed in patients with bacteriuria and/or leucocyturia of doubtful significance diagnosed by standard methods i e urine obtained as clean voided bag specimens in infants or as clean voided mid stream specimens in children

In a total number of 120 patients a clean voided urine specimen was obtained simultaneously with or immediately after performing a suprapubic bladder puncture The age and sex of the patients studied as well as the indication for the investigation within each age group is presented in Table 1

Technique of urine sampling

In infants urine was collected after feeding while in children it was obtained as the first morning sample

Percutaneous suprapubic aspiration of urine (SPA) This procedure was carried out after cleansing the suprapubic area with alcohol and iodine In children we used a spinal needle (type Yale 22 G 1 1/2 in) and in infants a No 25 G 1 in needle In children local anaesthesia with chlorethyl spray was applied The needle was inserted vertically through the abdominal wall in the midline 1 to 2 cm above the symphysis pubis and the bladder was punctured with a rapid stabbing movement The urine was gently aspirated in a 5 or 10 cc sterile syringe and the needle withdrawn

Clean voided urine In infants the sample was collected in a sterile polyethylene urine bag (Hollister® U BAG) after previous proper cleansing of vulva and preputial folds and perineum Irrigation of vulva and prepuce respectively was performed twice with 5 to 10 ml tepid physiologic saline Watch was kept so that the specimen was collected and chilled immediately after micturation In children a clean voided midstream specimen was obtained after thorough cleansing as described above

Immediately after collection the samples were chilled and transported to the laboratory for bacterial culture Standard bacterial technique was used which allowed detection of 10 or more bact/ml urine *Urinary infection was considered to be present if the bacterial count was $>10^{0}$/ml in SPA urine (= true bacteriuria)* and was suspected if the bacterial count in clean voided urine was >10 /ml Shortly after

Table 1 *Clinical material*

Indications for suprapubic aspiration (SPA)	Sex	Group A (infants) 0–1 month	Group A (infants) 1–18 months	Group B (children) 3–12 years	
Suspected urinary tract infection	boys	32	13	4	
	girls	28	11	12	
Myelomeningo-cele	boys	—	—	7	
	girls	—	2	4	
Glomerulo-nephritis	boys	—	—	2	
	girls	—	—	5	
Total		60	26	34	= 120

collection the uncentrifuged urine was also examined for pyuria expressed as leucocyte count per mm³ using a Burker counting chamber (8, 9). Leucocyte counts of >10 cells/mm³ in SPA urine was considered pathological (6). As to the limit for leucocyte count in clean voided urine, see Results.

RESULTS

Urinary infection, i.e. true bacteriuria in SPA urine, was found in group A (infants) in 19 neonates (0–1 month), 18 boys and 1 girl, and 5 infants (1–18 months), 2 boys and 3 girls. In group B (children) there was a female preponderance of urinary infection with 4 boys and 10 girls. The incidence of urinary infection was thus in the neonatal period highest among boys and in later childhood highest among girls.

Bacterial counts

Comparison of bacterial counts in SPA and concomitantly obtained clean voided urine. As shown in Table 2, in group A (infants) the suspicion of infection, i.e. bacterial count $>10^5$/ml in clean voided urine, could be excluded by normal SPA urine in 27 babies. On the other hand 4 babies had infection in SPA urine despite only slight or moderate bacteriuria in clean voided urine. The degree of contamination in clean voided urine samples with bacterial count $>10^5$/ml was lower among boys (13 versus 17) than among girls (14 versus 3).

In group B (children) 4 patients with suspected infection in clean voided urine proved to be free of infection by examination of the SPA urine, whereas 4 patients with true bacteriuria had a low bacterial count in voided urine. Misleading information about bladder urine bacteriuria was thus obtained from bacterial culture of clean voided specimens in 39 of 120 examined patients.

Table 2 *Bacterial counts per ml clean voided urine in relation to SPA urine*

Clean voided urine	SPA urine $<10^5$	♂/♀	SPA urine $>10^5$	♂/♀
Group A				
$<10^5$	35	12/23	4	3/1
$>10^5$	27	13/14	20	17/3
Group B				
$<10^5$	16	7/9	4	1/3
$>10^5$	4	2/2	10	3/7

Leucocyte counts

Comparison of leucocyte counts in SPA and concomitantly obtained clean voided urine. In Fig. 1 *a* and Table 3 is shown that clean voided urine of infants (group A) contained in general a higher number of leucocytes than the simultaneously obtained SPA urine. When the leucocyte count in SPA urine was normal (≤ 10/mm³) leucocyte count of clean voided specimens was always below 250/mm³. On the contrary, a raised leucocyte count of >10/mm³ in SPA urine was, with only one exception, combined with a leucocyte count of >250/mm³ in clean voided urine.

In children (group B) the leucocyte count also showed a tendency towards higher values in clean voided as compared to SPA urine (Fig. 1 *b*), but this was less pronounced than in infants (group A). In line with this increased leucocyte counts were found in clean voided samples from only 4 children with normal leucocyte counts in SPA urine (see Table 3).

In conclusion, our findings confirm that contamination (judged by leucocyturia) of clean voided samples occurs more frequently among infants than among children. However, more than 250 leucocytes/mm³ in clean voided

urine should be considered pathological in infants

Diagnostic value of leucocyte counts

As shown in Table 4 and Fig 1 *a* in infants true bacteriuria was generally coupled with leucocyturia In all 20 infants with leucocyte counts >250/mm³ in clean voided urine infection was verified by SPA However 4 babies with urinary infection ascertained by SPA had leucocyte counts <250/mm³ in clean voided urine Only 1 of these 4 had leucocyturia in SPA urine

In children true bacteriuria was found only in the presence of leucocyturia >10/mm³ in clean voided urine but with one exception On the other hand abnormal leucocyturia in clean voided urine was also found in 9 children without infection e g in children with glomerulonephritis and myelomeningocele (see Table 4 and Fig 1 *b*)

In conclusion in infants leucocyte counts of >250/mm³ in clean voided and >10/mm³ in SPA urine always indicated infection whereas in children leucocyturia of >10/mm³ whether in clean voided or SPA urine was inconclusive

DISCUSSION

The method of suprapubic aspiration of urine (SPA) was introduced in clinical pediatrics in 1959 by Pryles et al (18) but it has not gained widespread use mainly because of doubts about the safety of the method Although slight microscopic hematuria is common gross hematuria is extremely rare (11) In a large collaborative study only one complication a child with early peritonitis, was reported after bladder punctures in 5 000 infants and children (6)

In our department about 500 bladder punctures have been performed without any undue complication The rate of failure has in our hands become less than 5%

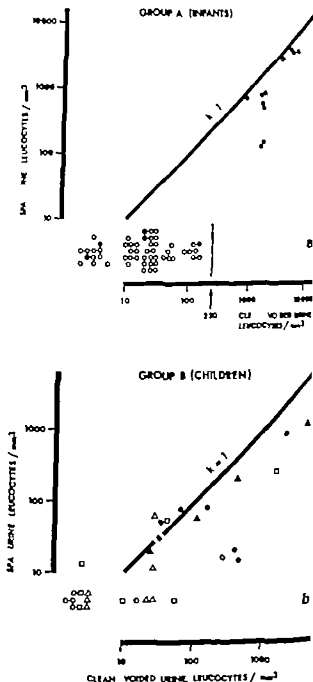


Fig 1 (*a b*) Comparison of leucocyte count in SPA urine and concomitantly obtained clean voided urine Suspected urinary infection (○) myelomeningocele (△) glomerulonephritis (□) Filled symbols (● ▲ ■) indicate infection proved by bacterial culture on SPA urine Skew lines ($k = 1$) identical leucocyte counts In Fig 1 *a* the vertical line at 250 leucocytes/mm³ indicates the maximal degree of contamination of clean voided urine in infants

Besides being an easily performed and safe method (15 16 18 19 22 23 24) we have found that it contributes significantly to the accuracy of diagnosing urinary tract infections

Table 3 *Leucocyte counts per mm³ clean voided urine in relation to SPA urine*

Clean voided urine	SPA urine	
	<10	>10
Group A		
<250	65*	1
>250	0	20
Group B		
<10	11	1
>10	4	18

* All neonates in this group had <5 leucocytes/mm

Bacterial counts

*Several investigations have shown the superior*ity of the suprapubic aspiration method for diagnosing true bacteriuria indicative of urinary infection in comparison to standard methods of urine collection (1 2 3 16 18 20 22 23 24) This is also apparent from the results of the present investigation of 120 infants and children False diagnosis was corrected after SPA in 39 cases 31 cases being false positive and 8 false negative in clean voided urine specimens Consequently the diagnosis urinary infection particularly in infants should not be based entirely upon bacterial culture of clean voided urine By performing SPA unnecessary drug therapy as well as laborious examinations of the urinary tract can be avoided

Leucocyte counts

Accurate bacterial counting is probably mandatory for diagnosing urinary infections (6 10

Table 4 *Leucocyte counts per mm³ in clean voided urine and SPA urine (within parentheses) in relation to bacterial counts (per ml) in SPA urine*

Leucocytes	SPA urine	
	$<10^5$	$>10^5$
Group A		
<250 (10)	6 (6.)	4 (3)
>250 (10)	0 (0)	20 (21)
Group B		
<10 (10)	11 (14)	1 (1)
>10 (>10)	9 (6)	13 (13)

12) whereas the value of leucocyte counting has been disputed (3 20 24)

In the present study the diagnostic implication of leucocyte counting was evaluated against the results of bacterial culture of SPA urine All normal infants had leucocyte counts <250/mm³ in clean voided and <10/mm³ in SPA urine In fact all normal neonates had <5/mm³ in SPA urine On the other hand leucocyturia of >250/mm³ and >10/mm³ in clean voided and SPA urine respectively was an almost constant finding in infants with urinary infection ascertained by true bacteriuria in SPA urine The only exception from this rule are infants with bacteriuria without leucocyturia In children the leucocyte counting was of less value for diagnosing urinary infection

In literature it has often been stated that bacteriuria might be present in the abscence of significant leucocyturia thereby depreciating the diagnostic value of leucocyte counting (3 20 24) In the present investigation however true bacteriuria without leucocyturia was found in only 4 infants and 1 child Thus in most cases low leucocyte count in clean voided urine will exclude urine infection As a consequence of our results we propose that a leucocyte count of 250/mm³ is a useful limit for evaluation of leucocyturia in clean voided bag specimens in infants Our results are in agreement with those published by other investigators (3 16 17) although some have suggested a somewhat higher (300 cells/mm³) or lower cell number (50 cells/mm³) as a limit in bag collected urine in infancy

In light of this urinary leucocyte counting should be re-evaluated as it is apparent that it is most helpful When being accurately performed contamination can be separated from infection in most cases

SUMMARY

In 120 infants and children a comparison was made between suprapubic aspiration urine and concomitantly obtained clean voided urine

urine should be considered pathological in infants

Diagnostic value of leucocyte counts

As shown in Table 4 and Fig 1 *a* in infants true bacteriuria was generally coupled with leucocyturia In all 20 infants with leucocyte counts >250/mm³ in clean voided urine, infection was verified by SPA However 4 babies with urinary infection ascertained by SPA had leucocyte counts <250/mm³ in clean voided urine Only 1 of these 4 had leucocyturia in SPA urine

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DISCUSSION

The method of suprapubic aspiration of urine (SPA) was introduced in clinical pediatrics in 1959 by Pryles et al (18) but it has not gained widespread use mainly because of doubts about the safety of the method Although slight microscopic hematuria is common gross hematuria is extremely rare (11) In a large collaborative study only one complication a child with early peritonitis was reported after bladder punctures in 5 000 infants and children (6)

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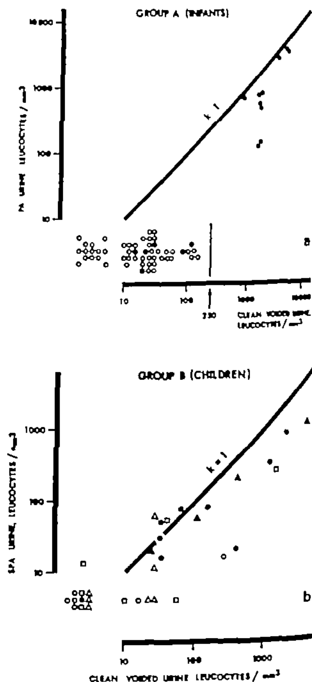


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Besides being an easily performed and safe method (15 16 18 19 22, 23 24) we have found that it contributes significantly to the accuracy of diagnosing urinary tract infections

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EVALUATION OF THE EFFECTIVENESS OF TREATMENT ON ADULT HEIGHT PROGNOSIS IN DISORDERS WITH ADVANCED AND RETARDED BONE AGE

Height and Height Velocity related to Chronological or to Bone Age

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The *final* adult height is a function of linear growth and skeletal maturation. In evaluating the efficiency of a given treatment which has the aim of improving adult height prognosis one must consider the *effect* of this *treatment* on both height velocity and skeletal maturation. Prediction of adult height with the *tables* of Bayley & Pinneau (1) is not possible in disorders which exhibit a severely pathological growth pattern because these tables are based upon longitudinal growth studies of normal children.

This communication offers alternative growth-related parameters and means of graphical presentation of growth data to be used for the evaluation of the effect of treatment on adult height prognosis. A case of precocious puberty and a case of hypopituitary growth retardation are used as examples.

PATIENTS AND METHODS

The child with precocious puberty is a boy with suprasellar hamartoma. Despite surgery the signs of precocious sexual development progressed further. He was started on Cyproterone acetate at the chronological age of 4 6 years (bone age 12 5, height age 7 [illegible] years). The boy with hypopituitary growth retardation was 7 2 years old when human growth hormone (HGH) treatment was started (bone age 3 6, height age 3 9 years). Height was measured using a Harpenden stadiometer. The standards used for height and height velocity are those of Tanner et al. (5). Bone age was assessed by the method of Tanner et al. (4). Chronological age (CA), height age (HA) and bone age (BA) are given in decimals of years.

PRESENTATION OF DATA AND INTERPRETATION

Height

In Fig. 1 the absolute height of the boy with precocious puberty is plotted with a thin line in the conventional way against chronological age. The physiologically more meaningful way, however, is to relate the linear growth to bone age (heavy line), as already proposed by Visser et al. (7). After the onset of therapy this curve is seen to be deflected toward the normal range. Cyproterone acetate has a slowing effect on skeletal maturation (2, 3). The consequence of this beneficial effect of the therapy on linear growth is not recognizable if height is plotted in the conventional way against chronological age.

The same two ways of plotting height are compared again in Fig. 2 for the boy with hypopituitary growth retardation. Although the plot against bone age is of physiological interest, only the conventional way of plotting height against chronological age demonstrates the beneficial effect of HGH treatment, since HGH exerts a similar stimulating effect upon

The following conclusions could be made from this study

(1) By performing suprapubic aspiration of urine true bacteriuria could be diagnosed 31 false positive and 8 false negative clean voided urine samples were disclosed

(2) Furthermore by the combined procedure of urinary collection it was possible to elucidate the diagnostic value of accurately performed leucocyte counting in uncentrifuged urine In infants leucocyte counts above 250/mm³ in clean voided urine and above 10/mm³ in suprapubic aspiration urine always accompanied a urinary infection whereas the degree of leucocyturia in children was inconclusive

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bone age (section *b*) it is apparent that this increase in height velocity occurs during the pubertal growth spurt It is therefore quite conceivable that this increase in height velocity might also have happened without treatment

In the case of hypopituitary growth retardation (Fig 4 section *a*) *it is of interest that in* the first year of HGH treatment height velocity reaches a supernormal level if it is plotted against chronological age In contrast when plotted against bone age (Fig 4 section *b*) it is seen to increase only into the normal range Most other children with hypopituitary *growth retardation exhibit a more pronounced* catch up growth than this boy height velocity exceeding the normal range in both ways of presentation

The most physiological way of presenting height velocity is to relate height increment

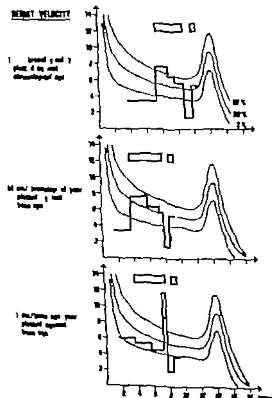


Fig 4 Different ways of calculating and plotting height velocity in a boy with hypopituitary growth retardation. For explanation see text.



Fig 5 Developmental quotient in a boy with precocious puberty prior to and during treatment For explanation see text.

to a bone age year and to plot this against bone age (section *c* of Figs 3 and 4) Subnormal values reflect a poor supernormal values a better adult height prognosis With this *presentation* it can be demonstrated that the boy with precocious puberty (Fig 3 *c*) had a deterioration of adult height prognosis prior to treatment With the institution of therapy however height velocity and in consequence adult height prognosis were improved This way of presentation however has a technical *limitation in the methodological accuracy of* reading bone age If bone age is progressing slowly as in hypopituitary growth retardation the difference of two bone age readings can be small When the actual measurements of height increment and bone age increment are transformed into the ratio height increment/one bone age year a relatively small error in bone age reading might be amplified For instance the increase of the height velocity (expressed as height increment to bone age year) at the end of the first period of the HGH treatment (Fig 4 *c*) can only be explained by the small change in bone age between the two readings This way of presenting height velocity is therefore only acceptable in diseases in which height and skeletal maturation increase with high speed such as in precocious puberty

The relating of height increment to bone age increment (Fig 3 *c* and 4 *c*) can also be presented as a developmental quotient ΔHA/ΔBA as shown in Fig 5 Normally this quotient *should* always be *1* even during the pubertal growth spurt a quotient below 1 indicates a *deterioration of adult* height prognosis a quotient of over 1 an improvement Thus

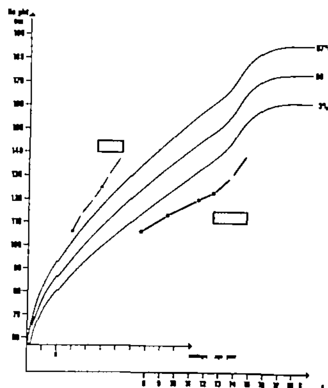


Fig 1 Height of a boy with precocious puberty plotted against both chronological age (thin line) and bone age (heavy line) prior to and during treatment (hatched area) For further explanation see text

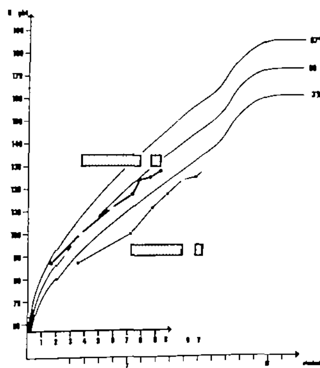


Fig 2 Height of a boy with hypopituitary growth retardation plotted against both chronological age (thin line) and bone age (heavy line) prior to and during treatment (hatched area) For further explanation see text

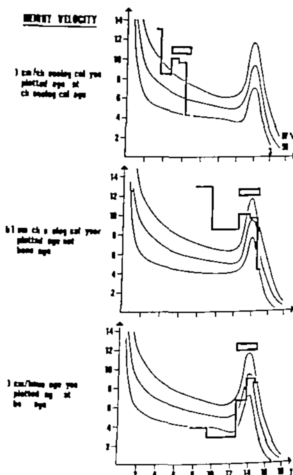


Fig 3 Different ways of calculating and plotting height velocity in a boy with precocious puberty For explanation see text

both height increment and bone age increment

Height velocity

The conventional way of presenting height velocity is to give the height increment per chronological year and to plot this increment against chronological age (section *a* of Figs 3 and 4) Since the onset of the pubertal growth spurt is closely related to bone age (6) a more meaningful presentation can be achieved if height velocity (height increment/chronological year) is plotted against bone age (section *b* of Figs 3 and 4) In the case of the boy with precocious puberty treated with Cyproterone acetate (Fig 3) the conventional presentation (section *a*) would suggest that the increase of height velocity is due to the treatment If however the same data are plotted against

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IMPROVEMENT OF ADULT HEIGHT PROGNOSIS IN PRECOCIOUS PUBERTY BY CYPROTERONE ACETATE

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Various treatments have been used to improve adult height prognosis in precocious puberty Antigonadotropic treatment with progestational agents has been introduced by Richie & Crawford (25) and Greenblatt et al (8) These authors used 17 alpha-ethinyl 19 nortestosterone No clearcut effect on skeletal maturation was found In 1962 Kuppermann & Epstein (17) suggested medroxyprogesterone acetate for the treatment of idiopathic precocious puberty Several authors reported no clearcut effect on skeletal maturation (7 11 15 26 32 33 34) In 1971 Greenblatt et al (9) reported on seven children they treated with the antigonadotropin Danazol a synthetic (2 3 isoxazol) derivative of 17 alpha ethinyl testosterone An effect on skeletal maturation seems to have occurred In 1963 Neumann & Hamada (20) presented a new antiandrogenic and progestational compound Cyproterone acetate (1 2 alpha methylene 6-chloro-4 6 pregnandiene 17 alpha-ol 3,20-dione 17 alpha acetate SH 714 of Schering Co Berlin) with evidence for an antigonadotropic action The limited reports on the use of this drug in precocious puberty (3 12) revealed discrepancies concerning skeletal maturation and adult height prognosis By application of different growth and maturation parameters we intend to demonstrate an improvement of adult height prognosis in precocious puberty by Cyproterone acetate

PATIENTS AND METHODS

The clinical data of the patients are presented in Table 1 Cyproterone acetate was administered at the dosage of 60 mg/m /day in three divided doses The clinical signs of puberty were rated according to Tanner (28) The testicular volume was measured using the orchidometer of Prader (24) intermediate testicular sizes were interpolated Height was measured using the Harpenden stadiometer All bone ages were estimated by one of us (E B) according to the method of Tanner et al (29) Chronological age (CA) height age (HA) and bone age (BA) are given in decimals of years The data of growth and skeletal maturation are presented as described elsewhere (4) Urinary gonadotropins were measured by the mouse uterine assay of Johnsen (13) and total urinary estrogen excretion by a modification of the methods of Scholler et al (27) and Brown et al (5) Papanicolaou smears were performed on vaginal smears or on exfoliated cells in urine according to Collet Solberg & Grumbach (6) 17 ketosteroids were measured by the method of Bean et al (2) At each visit, serum bilirubin and GOT GPT and alkaline phosphatase activities were measured

RESULTS

The effect of Cyproterone acetate on the signs of puberty can be seen in Table 1 Pubic hair regressed or remained sparce despite advanced bone age No axillary hair developed during therapy Testicular volume showed an initial regression and a subsequent increase with skeletal maturation reaching peak pubertal stages Breast size decreased in girls Menstruation ceased in patient B V and did not occur in patient D R. despite a bone age of 14.9 Patient T T had one single vaginal

the beneficial effect of Cyproterone acetate on the adult height prognosis of this boy with precocious puberty is demonstrated

SUMMARY

Different growth parameters and their graphical presentation in disorders with advanced and retarded bone age are discussed Their usefulness for the evaluation of the effect of treatment on growth and maturation is compared In precocious puberty more information is obtained when height is plotted against bone age instead of chronological age It is more reasonable from a physiological standpoint to plot height velocity against bone age rather than chronological age For the evaluation of the efficiency of treatment on adult height prognosis in precocious puberty two possibilities are proposed height velocity expressed as height increment per bone age year or the quotient height age increment/bone age increment However where skeletal maturation is severely retarded, as in hypopituitary growth retardation these growth parameters should be related in the conventional way to chronological age rather than to bone age

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Table 2 Urinary gonadotropins 17 ketosteroids total estrogens and Papanicolaou smears (percentage of acidophilic and basophilic karyopyknotic superficial cells)

	Before treatment				During treatment														
Years	−1	−0.5	−0.25	0	0.25	+0.5	+0.75	+1	+1.25	+1.5	+1.75	+2	+2.25	+2.5	+2.75	+3	+3.25	+3.5	+3.75
Gonadotropins (U/24 hrs)																			
T T						23	14	38	32	14	34	24							
B V			0	0	<8	18		<8											
D R				35		45	<16	37	18	29	23								
E W	<10											13		0	13	<11	[illegible]	9	20
P T		<12	<5		<5		13			0									
17 KS (mg/24 hrs)																			
T T						1.0	0.7	1.7	1.2	0.9	0.1	8	3.1	2.0					
B V			0.9	0.9		4.5	0.7	1.1	2.7	1.3									
D R				1.0		1.5		2.4	2.1	1.4	2.2		1.2						
E W	1	1.5	0.8	1.1								2.8		2.0	7.9	3.0	2.2		0.7
P T		0.7	1.0		[illegible]		1.4			1.9									
Total Estrogens (µg/24 hrs)																			
T T								5.3	4.1	1.8	0.1		8	8.2	2.0				
B V				2.2		1.9			2.7	1.3		6.8							
D R				4				4.2	0.4										
Papanicolaou smears																			
T T						18	1	5	5	6	0	0	0	0	0				
B V			21	1	0	0	1												
D R				11	0	0	2		2		0								

Table 1 *Clinical data of patients with precocious puberty Effect of Cyproterone acetate treatment on signs of puberty*

Patient	Onset of therapy							Last visit							
	CA (y)	BA (y)	Height (cm)	HA (y)	Breasts (1–5)	Pubic hair (1–6)	Testic Vol (ml)	CA	BA	Height	HA	Breasts	Pubic hair	Testic Vol	Particularities
T T ♀	2 9		108 0	5 1	2	2		5 6	12 7	132 0	9 2	1	1		Single vaginal spotting 2 years after onset of therapy No spotting or menstruation after increase of dose
B V ♀	4 3	5 7	109 8	5 3	2	2		5 6	8 9	119 4	7 0	1	1		2 menstruations prior to therapy no recurrence since treatment
D R ♀	6 6	12 7	136 0	9 9	3	2		9 0	14 7	147 0	11 6	2	1		No menstruation despite BA of 14 7
E W ♂	8 4	13 8	149 0	12 4		1	18/16	11 7	16 8	172 4	16 2		2	15/15	Initial reduction of testicular volume to 11/10 ml under treatment
P T ♂	4 6	12 5	122 0	7 2		4	15/12	6 6	14 8	138 1	10 2		4	12/12	Suprasellar hamartoma operated at age 3 06 without effect on progression of precocious puberty Initial reduction of testicular volume to 12/8 under treatment

Table 2 *Urinary gonadotropins 17 ketosteroids Total estrogens and Papanicolaou smears (percentage of acidophilic and [illegible] notic superficial cells)*

	Before treatment				During treatment														
Years	−1	−0 5	−0 25	0	+0 25	+0 5	+0 75	+1	+1 25	1 5	+1 75	+2	+2 25	+2 5	+2 75	+3	+3 25	+3 5	+3 75
Gonadotropins (U/ 4 hrs)																			
T T						21	14	38	32	14	34	24							
B V			0	0	<8	18		<8											
D R				35		45	<16	37	18	79	23								
E W	<10											13		0	13	<11	12	29	70
P T		<12	<5		<5		13			0									
17 KS (mg/24 hrs)																			
T T						1 0	0 7	1 7	1 2	0 9	0 1	2 8	3 1	2 0					
B V			0 9	0 9		4 5	0 7	1 1	2 7	1 3									
D R				1 0		1 5		2 4	2 1	1 4	2 2		1 2						
E W	1 7	1 5	0 8	1 1								2 8		2 0	7 9	3 0	2 2		0 7
P T		0 7	1 0		1 3		1 4			1 9									
Total Estrogens (μg/24 hrs)																			
T T								5 3	4 1	1 8	0 1		2 8	8 2	2 0				
B V				2 2		1 9			2 7	1 3		6 8							
D R				4				4 2	0 4										
Papanicolaou smears																			
T T						18	1	5	5	6	0	0	0	0	0				
B V			21	1	0	0	1												
D R				11	0	0	2		2		0								

Table 1 *Clinical data of patients with precocious puberty Effect of Cyproterone acetate treatment on signs of puberty*

Patient	Onset of therapy CA (y)	BA (y)	Height (cm)	HA (y)	Breasts (1–5)	Pubic hair (1–6)	Testic Vol (ml)	Last visit CA	BA	Height	HA	Breasts	Pubic hair	Testic Vol	Particularities
T T ♀	2 9		108 0	5 1	2	2		5 6	12 7	132 0	9 2	1	1		Single vaginal spotting 2 years after onset of therapy No spotting or menstruation after increase of dose
B V ♀	4 3	5 7	109 8	5 3	2	2		5 6	8 9	119 4	7 0	1	1		2 menstruations prior to therapy no recurrence since treatment
D R ♀	6 6	12 7	136 0	9 9	3	2		9 0	14 7	147 0	11 6	2	1		No menstruation despite BA of 14 7
E W ♂	8 4	13 8	149 0	12 4		1	18/16	11 7	16 8	172 4	16 2		2	15/15	Initial reduction of testicular volume to 11/10 ml under treatment
P T ♂	4 6	12 5	122 0	7 2		4	15/12	6 6	14 8	138 1	10 2		4	12/12	Suprasellar hamartoma operated at age 3 06 without effect on progression of precocious puberty Initial reduction of testicular volume to 12/8 under treatment

Table 2 *Urinary gonadotropins 17 ketosteroids total estrogens and Papanicolaou smears (percentage of acidophilic and basophilic karyopyknotic superficial cells)*

	Before treatment				During treatment														
Years	−1	−0 5	−0 5 0		+0 25	+0 5	+0 75	+1	+1 25	+1 5	+1 75	+2	+2 25	+2 5	+2 75	+3	+3 25	+3 5	+3 75
Gonadotropins (U/24 hrs)																			
T T						23	14	38	32	14	34	24							
B V			0	0	<8	18		<8											
D R				35		45	<16	37	18	29	23								
E W	<10											13		0	13	<11	12	29	40
P T		<12	<5		<5		13			0									
17 KS (mg/24 hrs)																			
T T						1 0	0 7	1 7	1 2	0 9	0 1	2 8	3 1	2 0					
B V			0 9	0 9		4 5	0 7	1 1	2 7	1 3									
D R				1 0		1 5		2 4	2 1	1 4	2 2		1 2						
E W	1 7	1 5	0 8	1 1								2 8		2 0	7 9	3 0	2 2		0 7
P T		0 7	1 0		1 3		1 4			1 9									
Total Estrogens (µg/24 hrs)																			
T T								5 3	4 1	1 8	0 1		8	8 2	2 0				
B V				2 2		1 9			2 7	1 3		6 8							
D R				4				4 2	0 4										
Papanicolaou smears																			
T T						18	1	5	5	6	0	0	0	0	0				
B V			21	1	0	0	1												
D R				11	0	0	2		2		0								

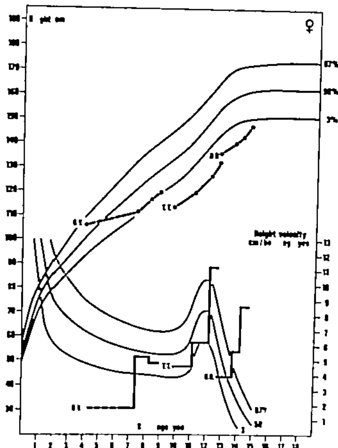


Fig 1 Improvement of both linear growth and height velocity related to bone age in three girls (be fore — during treatment)

spotting after the dose of Cyproterone acetate was doubled no spotting or menstruation ap peared although bone age was 12 7 years

Table 2 shows that there was no significant change in urinary excretion of gonadotropins 17 ketosteroids and total estrogens under Cyproterone acetate treatment Basophilic karyopyknotic superficial cells practically dis appeared from the Papanicolaou smears

Height, plotted against bone age tends to resume normality (patients T T D R and P T) or is prevented from further decline (pa tients B V and E W) with institution of Cyproterone acetate therapy (Fig 1 for girls and Fig 2 for boys) Accordingly height ve locity calculated as height increment/bone age year is increased in all children

In Fig 3 a developmental quotient, de fined by the ratio of height age increment over bone age increment (ΔHA/ΔBA) during a definite period of time, is presented All pa tients show a clear increase of this quotient under treatment height age now progressing faster relatively to bone age In patient E W ΔHA/ΔBA was already above 1 prior to treat ment but showed a marked increase during a prolonged period of 23 months It can at least be concluded that adult height prognosis did not deteriorate in this boy When last seen he measured 172 4 cm (Table 1)

No hepatotoxicity has been found in our pa tients as assessed by repeated measurements of bilirubin and GOT GPT and alcaline phos phatase activities Hematologic examinations were always normal The inhibitory effect on spermatogenesis was shown to be reversible after withdrawal of the drug both in rats (21) and in men (18 22 23)

DISCUSSION

Cyproterone acetate exerted a clear effect on the clinical signs of puberty which all re

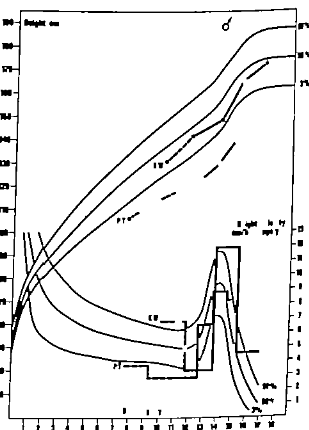


Fig 2 Improvement of both linear growth and height velocity related to bone age in two boys (be fore — during treatment)

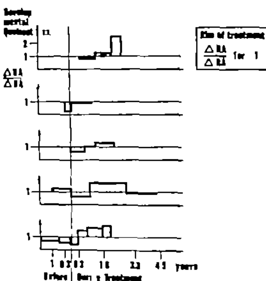


Fig. 3. Increase of the developmental quotient indicating improvement of adult height prognosis under Cyproterone acetate. The decrease of the developmental quotient to 1 toward the end of treatment in patient E. W. can be explained by the almost completed epiphyseal closure at that time (bone age 16.8 years).

gressed (Table 1). This is in agreement with the findings of Bierich (3) and Helge et al. (12). This effect was also found with 17 alpha ethinyl nortestosterone (8, 25), with medroxyprogesteroneacetate (7, 11, 15, 26, 34) and with the 2,3 isoxozol derivative of 17 alpha-ethinyl testosterone (9). The regression of pubic hair and the lack of appearance of axillary hair under treatment can be explained by the peripheral antiandrogenic effect of Cyproterone acetate. The regression of breast size and of the estrogenic effect on the vaginal epithelium must be related to an inhibition of gonadotropin secretion (14, 19, 33), although no decrease in urinary gonadotropins or estrogens could be measured (Table 2). Both assays might not be sensitive enough to reflect small changes which might still be biologically significant. Menstruation ceased in patient B. V. and did not appear despite advanced bone age in patients T. T. and D. R. This effect might not be attributable to inhibition of gonadotropin release alone, since the drug could also influence the uterine mucosa directly through its progestational effect. The testicular volume decreased initially under treatment. As skeletal maturation proceeded to peak pubertal stages, however, the testicles increased in size again. It may be assumed that the pubertal increase of gonadotropin release is not sufficiently suppressed by the Cyproterone acetate dosage used, while the peripheral antiandrogenic effect still prevents excessive skeletal maturation. Such a mechanism would be compatible with previous findings in rats, where the age (14) and the dosage of the drug (19) were shown to be of importance in the inhibition of gonadotropin secretion by Cyproterone acetate, and by findings in men, where this effect was observed to depend on pretreatment levels of gonadotropins (33).

The other important aim of the treatment of precocious puberty is to improve the adult height prognosis of the patients. Without treatment, adult height prognosis is poor. Only 4 out of 34 children in Thamdrup's series (31) achieved the height of the smaller of their parents. Helge et al. (12) reported a delaying effect upon skeletal maturation and a slowing effect upon growth velocity under Cyproterone acetate treatment. The decline of growth velocity was not optimal in the cases reported by Bierich (3). These two authors made no definite statement on adult height prognosis. By the use of different parameters for maturation and growth (as described elsewhere (4)) we were able to demonstrate an improvement of adult height prognosis by prolonged administration of Cyproterone acetate in all our patients. Height velocity, as defined by height increment/bone age year, was enhanced (Figs. 1 and 2) in our children. This effect of the treatment can be explained either by a stimulation of height velocity or by an inhibitory effect on skeletal maturation, or both. These possibilities cannot be distinguished in the single cases. The final effect on adult height prognosis, however, is the same.

The same data can be presented by the use of a developmental quotient, which eliminates

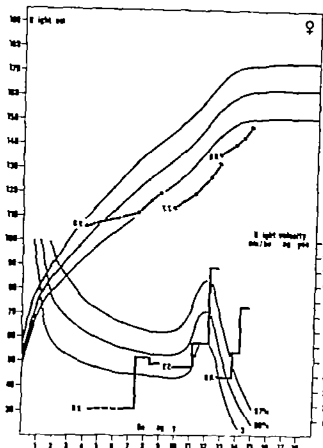


Fig 1 Improvement of both linear growth and height velocity related to bone age in three girls (before — during treatment)

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Table 2 shows that there was no significant change in urinary excretion of gonadotropins 17 ketosteroids and total estrogens under Cyproterone acetate treatment Basophilic karyopyknotic superficial cells practically disappeared from the Papanicolaou smears

Height, plotted against bone age tends to resume normality (patients T T D R and P T) or is prevented from further decline (patients B V and E W) with institution of Cyproterone acetate therapy (Fig 1 for girls and Fig 2 for boys) Accordingly height velocity calculated as height increment/bone age year is increased in all children

In Fig 3 a developmental quotient, defined by the ratio of height age increment over bone age increment (ΔHA/ΔBA) during a definite period of time, is presented All patients show a clear increase of this quotient under treatment, height age now progressing faster relatively to bone age In patient E W ΔHA/ΔBA was already above 1 prior to treatment but showed a marked increase during a prolonged period of 23 months It can at least be concluded that adult height prognosis did not deteriorate in this boy When last seen he measured 172 4 cm (Table 1)

No hepatotoxicity has been found in our patients as assessed by repeated measurements of bilirubin and GOT GPT and alcaline phosphatase activities Hematologic examinations were always normal The inhibitory effect on spermatogenesis was shown to be reversible after withdrawal of the drug both in rats (21) and in men (18 22 23)

DISCUSSION

Cyproterone acetate exerted a clear effect on the clinical signs of puberty which all re

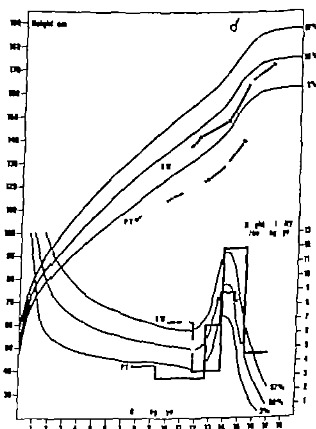


Fig 2 Improvement of both linear growth and height velocity related to bone age in two boys (before — during treatment)

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Key words Precocious puberty Cyproterone acetate adult height prognosis height velocity skeletal maturation

The Editorial Board has asked Dr Patrick Oun to comment on these two articles

E Bona E E Joss and R P Zurbrugg suggest in two interesting articles (2 3) new parameters for the evaluation of adult height prognosis in patients with advanced skeletal age and accelerated linear growth The second article concludes that the new antiandrogen Cyproterone acetate improves adult height prognosis in precocious puberty

It is well known that the methods hitherto used for such prognosis i e the tables of Bayley & Pinneau are far from accurate Follow up studies are difficult to interpret since

the possible interference of the pubertal growth spurt with the significance of the results (4) and which comprises the effects of both the above mentioned mechanisms This quotient is defined as the ratio between increment of height age to increment of skeletal age (ΔHA/ΔBA) during a definite period of time Adult height prognosis is considered to be improved where the developmental quotient is increased An accurate prediction of adult height prognosis cannot be made by the use of the tables of Bayley & Pinneau (1) in precocious puberty since these tables are the result of data on growth patterns of normal children and are not relevant for pathological growth patterns Furthermore they can only be used if the bone age is determined by the method of Greulich & Pyle (10) We preferred the method of Tanner et al (29) because of its greater accuracy The developmental quotient has clearly been increased in our patients (Fig 3) Thus the adult height prognosis of our children with precocious puberty has been improved by the use of Cyproterone acetate

SUMMARY

Three girls and one boy with idiopathic precocious puberty and one boy with operated suprasellar hamartoma and progressing precocious puberty were treated with Cyproterone acetate for periods ranging from 15 months to 4 years and 6 months In all children the signs of puberty were reduced Height velocity as related to bone age was increased as was the ratio of height age increment to bone age increment An improvement of adult height prognosis in precocious puberty has been achieved by Cyproterone acetate

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The authors are grateful to Dr A Engelborn Laboratoire d Hormonologie Maternite Geneve for carrying out the measurements of urinary gonadotropins and to Dr E Lauber Central Chemical Laboratories of the Inselspital Berne for performing the measurements of urinary estrogens They owe the Cyproterone acetate (SH 714) to Schering Co Berlin

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COLLECTIVE RESULTS OF MASS SCREENING FOR INBORN METABOLIC ERRORS IN EIGHT EUROPEAN COUNTRIES

At the invitation of the Public Health Committee of the Council of Europe a working party was set up in 1970 to gath r information on newborn screening for inborn errors of metabolism in nine European countries The delegates were asked to collect the results from the commencement of screening programmes in their countries till the year 1970 or 1971 respectively The most important data of this survey concerning errors of aminoacid and galactose metabolism will be presented in this paper It must be pointed out that the material is non homogeneous the beginning of mass screening differed as did the test methods and the evaluation of their results Guthrie s microbiological inhibition assay paper and thin layer chromatography fluorimetry (for phenylketonuria) and Beutler s enzyme test (for galactosaemia) were the principal screening methods performed almost exclusively on capillary blood Over 90 of the results for phenylketonuria maple syrup urine disease and homocystinuria were obtained by the Guthrie technique The participating countries and the screening periods for eight European countries are listed in Table 1

The figures presented in Table 2 refer to *classical phenylketonuria* (PKU) in contrast to other forms of hyperphenylalaninaemia It is often difficult to distinguish between these various forms their definition varying to some extent from country to country

As a rule classical PKU had to fulfil the following criteria

(1) a blood phenylalanine level persistently above 20 mg on a normal protein intake after the first week of life

(2) a normal level of tyrosine and of the other aminoacids in blood

(3) a characteristic result of the oral phenylalanine loading test

(4) phenylketones may be present in the urine

As shown in Table 2 the total of newborns tested was 5 252 000 of whom 668 showed classical PKU The frequency of PKU in this newborn population was therefore about 1 8 000 The incidence rate varies considerably in different countries Thus PKU seems to be more frequent in Ireland and in the Federal Republic of Germany than in Switzerland These variations are at present difficult to explain They may partly be due to genetic differences between the countries but they may also be influenced by different definitions of classical PKU Furthermore in this and still more in the following tables the error inherent in the limited number contributes significantly to these variations

The total test frequency in the eight European countries during the reported period was about 53 After some years when the screening programme has become well established in a country the coverage of the newborn population improves greatly this is borne out by the recent screening results for Switzerland in 1971 which covered 97 % of all newborns as well as for the Federal Republic of Germany with coverage of 87 and Denmark with coverage of 88

Concerning the screening for *maple syrup urine disease* (MSUD) much less data is available

Table 3 shows the total sum of 1 5 million

the institution of treatment blocks the possibility to know how the patient would grow without treatment One is left with comparisons with other patients often studied by other investigators with different methods

In the present papers an attempt has been made to increase the accuracy of the prognosis by utilizing bone age according to Tanner related to height velocity per bone age year and a developmental quotient of Δheight age/Δbone age and comparing pretreatment growth with growth during prolonged treatment in the five patients studied Does this design increase the accuracy of the adult height prognosis? It might but the authors have not shown that the height velocity in cm/bone age year is changed in a consistent way by the treatment (see Fig 1 and 2 (3)) in fact in two patients pretreatment values are lacking and in one of the remaining three pretreatment growth is adequate

The second parameter used the developmental quotient, should always be 1 in the normal child according to the authors (2) They do not consider a normal variation around this norm or mean nor do they consider the standard error of the mean What deviation from 1 should be considered significant? Which methodological factors influence the value of the developmental quotient? The systematic error of bone age measurements according to Greulich Pyle and Tanner Whitehouse is about 4 months or 0 3 years as reported by Andersen (1) This error naturally must influence the developmental quotient rather much especially when the Δbone age is close to or less than one year These are questions that have to be considered before the developmental quotient can be utilized as a reliable guide to changes in adult height prognosis The conclusion of the article on the improvement of adult height prognosis in precocious puberty by cyproterone acetate cannot be taken for granted until further studies including follow up to adult height have been completed

Patrick Olin

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Table 4 *Screening results for homocystinuria*

Country	Live births	Newborn tests		Path	Ratio
Belgium	141 119	3 146	2	0	
Denmark	554 100		0		
Fed Rep Germany	2 525 032	621 654	25	2	1 310 827
France	3 193 607	209 347	7	2	1 104 674
Great Britain	1 824 162	550 324	30	2	1 275 162
Ireland	363 351		0		
Netherlands	604 156		0		
Switzerland	731 953	303 913	4	0	
Total	9 937 481	1 688 384	17	6	1 281 397

Table 6 *Screening results for histidinemia*

Country	Live births	Newborn tests		Path	Ratio
Belgium	141 119	3 146	2	0	
Denmark	554 100		0		
Fed Rep Germany	2 525 032		0		
France	3 193 607	91 181	3	2	1 45 590
Great Britain	1 824 163	120 376	7	16	1 7 524
Ireland	363 351		0		
Netherlands	604 156		0		
Switzerland	731 953		0		
Total	9 937 481	214 703	2	18	1 11 972

in populations with a high incidence of the corresponding genes

Recommendations for screening programmes

These recommendations are influenced by the availability of effective treatment From the evidence presented in Tables 2–7 routine newborn screening is recommended without reservation for PKU MSUD galactosemia and histidinemia Some reservations exist concerning the screening for homocystinuria because of questionable reliability and insufficient data and for tyrosinemia because of the overwhelming number of transient forms reported Despite the reservations the inclusion of these diseases in screening programmes is recommended in order to gain more knowledge about them

As regards the choice of the most suitable technique for screening errors of aminoacid and galactose metabolism the Guthrie tests have proved to be useful and are most widely applied They have the advantage of testing the afore mentioned diseases from a few drops of blood They are economical and a large number of tests can be performed by a relatively small and inexperienced staff These advantages outnumber certain disadvantages in the testing for galactosemia and homocystinuria Some laboratories with previous experience in thin layer and paper chromatography have successfully applied these techniques to screening programmes Chromatographic methods have the advantage of detecting many aminoacids in one single run They do however require more experience especially in interpreting the results and are more time-consuming and expensive

For G6PD deficiency at least all male newborn babies should be tested in selected regions The test of Motulsky and Campbell

Table 5 *Screening results for galactosemia*

Country	Live births	Newborn tests		Path	Ratio
Belgium	141 119		0		
Denmark	554 100		0		
Fed Rep Germany	2 525 032	373 537	15	9	1 41 726
France	3 193 607	10 934	1/2	2	1 5 467
Great Britain	1 824 163	79 906	4	2	1 39 953
Ireland	363 351		0		
Netherlands	604 156		0		
Switzerland	731 953	259 480	35	8	1 32 435
Total	9 937 481	725 857	7	21	1 34 565

Table 7 *Screening results for tyrosinosis* (for explanation see text)

Country	Live births	Newborn tests		Path	Ratio
Belgium	141 119	103 302	73	0 82	
Denmark	554 100		0		
Fed Rep Germany	2 525 032		0		
France	3 193 607	226 957	7	0,465	
Great Britain	1 824 163	254 667	14	1/107	1 254 667
Ireland	363 351		0		
Netherlands	604 156		0		
Switzerland	731 953		0		
Total	9 937 481	584 926	6	1/654	1 584 926

Table 1 *Screening period of eight European countries represented in the Council of Europe*

	From	To
Belgium	January 1970	December 1970
Denmark	January 1964	December 1970
Fed Rep Germany	January 1969	December 1971*
France	May 1967	February 1971
Great Britain	January 1969	December 1970
Ireland	February 1966	October 1971
Netherlands	January 1968	June 1970
Switzerland	January 1965	December 1971*

* Preliminary newborn numbers for 1971

babies tested 12 cases with suspected MSUD were verified by colomn chromatography This makes a provisional ratio of 1 121 000 as the size of the screening sample is still much too small Care must be taken not to miss infants who because of an early start of this very progressive disease may be transferred to a children s hospital and may there escape the routine screening programmes of maternity hospitals

Six of 1 7 million newborns screened for *homocystinuria* showed persistently elevated methionine blood levels (Table 4)

This result should be regarded with even greater reservation Some patients with homo cystinuria have been observed who developed a significant methionine increase only some weeks after birth thus that they would be missed by an early screening programme

On the other hand elevated methionine levels in the newborn age group can be due to

Table 2 *Screening results for phenylketonuria*

Country	Live births	Newb tests		Path	Ratio
Belgium	141 119	85 985	61	7	1 12 285
Denmark	554 100	187 000	30	15	1 12 466
Fed Rep Germany	2 525 032	2 049 589	81	340	1 6 028
France	3 193 607	971 944	30	77	1 12 622
Great Britain	1 824 163	1 157 556	63	143	1 8 094
Ireland	363 351	320 345	90	60	1 5 339
Netherlands	604 156	51 608	9	2	1 25 804
Switzerland	731 953	428 113	59	24	1 17 838
Total	9 937 481	5 252 140	53	668	1 7 862

Table 3 *Screening results for maple syrup urine disease (MSUD)*

Country	Live births	Newborn tests		Path	Ratio
Belgium	141 119	13 431	10	1	1 13 431
Denmark	554 100		0		
Fed Rep Germany	2 525 032	778 018	31	5	1 155 604
France	3 193 607	11 331	1/2	1	1 11 331
Great Britain	1 824 163	254 667	14	0	
Ireland	363 351		0		
Netherlands	604 156		0		
Switzerland	731 953	402 311	55	5	1 80 467
Total	9 937 481	1 459 758	15	12	1 121 647

a variety of causes such as liver diseases in cluding atresia of the bile duct hyperbilirubinaemia and other metabolic errors such as fructose intolerance and tyrosinosis

Screening for *galactosemia* has so far been been performed in only 725 000 newborns (Table 5) 3 of the 8 countries use the galactose test with the *E coli* mutant, whilst some other laboratories have recently started routine screening for uridyltransferase deficiency with the Beutler method There is no final agree ment as to which of these techniques is pre ferable In Switzerland both Coli and Beutler tests are used together in almost all newborns The incidence in the four countries performing any galactose testing is about 1 35 000

Screening experience for *histidinemia* in Western Europe is even scantier (Table 6) though after PKU this seems to be the second most frequent aminoacidopathy with brain damage The ratio of 1 12 000 agrees well with a similar figure of 1 18 000 reported from Austria by Thalhammer and co workers

Finally only preliminary screening data are so far available for *tyrosinosis* (Table 7)

In about 600 000 tests there was only one child with hereditary tyrosinosis The other 654 instances of tyrosinaemia were transient non hereditary metabolic deviations

Data on the diffusion of G 6 PD deficiency and thalassemia were collected in some count ries and the working party agreed on the opportunity of mass screening for those defects

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PERINATAL ACIDOSIS AND PLACENTAL TRANSFUSION[1]

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It has been suggested that fetal asphyxia results in a transfer of blood *in utero* from placenta to fetus Yao et al (11) studying infants born by Caesarean section found increased circulating blood volumes in those infants delivered after evidence of fetal distress Philip et al (7) studying infants born vaginally found reduced residual placental blood volumes (RPBV) despite early clamping in infants whose births were complicated by signs of fetal distress Both these studies were based entirely on clinical evidence of fetal distress Flod & Ackerman (3) studying umbilical artery blood pH values found a suggestive relationship between fetal acidosis and reduced RPBV

The present study was undertaken to further clarify the effect of fetal asphyxia on the distribution of blood volume between the placenta and fetus

MATERIAL AND METHODS

Seventy five term infants who were born vaginally were studied The umbilical cords were clamped as quickly as possible at birth and in all instances prior to the first breath A segment of cord was removed between two clamps for umbilical arterial blood sampling The arterial blood was drawn into a heparinized syringe which was then immediately placed in ice The blood was analysed for pH using a Radiometer micro blood gas apparatus

RPBV was measured as it was in a prior study (3) by using the method described earlier by Redmond et al (8) The RPBV was expressed in ml kg body weight of the infant

Thirty six of the infants were born to primiparous mothers Thirty nine of the infants were born to mothers whose parity ranged from 2 to 10 The median parity number for these 39 mothers was 4 The relationship between pH and RPBV was analysed separately for the primiparous and the multiparous deliveries

The mean duration of the second stage of labor was 32 minutes for the multiparous deliveries and 85 minutes for the primiparous deliveries

No instances of maternal diabetes were included in the present study Three mothers had preeclampsia

RESULTS

The mean umbilical artery pH was 7 28 ± 0 08 (range 7 07–7 47) for the multiparous deliveries and 7 22 ± 0 09 (range 6 97–7 38) for the primiparous deliveries The mean RPBV was 34 ± 13 ml/kg (range 18–85) and 31 ± 9 ml/kg (range 9–54) for infants of multiparous and primiparous mothers respectively

For infants of primiparous mothers acidosis was associated with clinical evidence of perinatal depression For infants of multiparous mothers in the present series a relationship was not apparent between pH and clinical status The relationship between reduced pH clinical status of the infant and RPBV is presented in Table 1

The relationship between pH and RPBV is illustrated for multiparous deliveries in Fig 1 and for primiparous deliveries in Fig 2

[1] Supported by Grant No PHS-RR05351 10 (GRS) National Institutes of Health Bethesda Maryland and a grant from Ross Laboratories Columbus Ohio

Kraut is the method of choice Testing for thalassemia trait is recommended in school age and in selected regions A determination of the osmotic fragility and possibly of A hemoglobin has to be carried out The test is not very important for the health of the child but in regions with high gene frequency of thalassemia the result is important for genetic counselling in order to prevent the serious disease of thalassemia major

The data of this short report were collected and prepared for publication by the following delegates of the Working Party of the Council of Europe to study Hereditary Metabolic Diseases R G Beckers (Belgium) E Wamberg (Denmark) H Bickel and E Schmid Ruter (Federal Republic of Germany), J Feingold (France) S F Cahalane (Ireland) E Bottini (Italy) J H P Jonxis (The Netherlands) J P Colombo (Switzerland) and N Carson (Great Britain)

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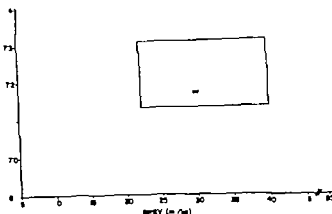


Fig 2 Umbilical artery blood pH and residual placental blood volume (RPBV) Primiparous deliveries The rectangular area includes all values within 1 S D of the mean for pH and RPBV The relationship between pH and RPBV was not statistically significant Note a cluster of values in the lower right portion of the rectangular area These values may represent infants with a brief duration of acidosis which did not cause a reduction of RPBV (see text)

et al (9) for infants with delayed clamping In a similar study Philip et al (7) suggested in infants born vaginally that fetal distress resulted in transfer of blood from placenta to infant during labor Flod & Ackerman (3) determined umbilical artery pH at birth and suggested that acidosis was related to increased transfer of blood *in utero* In two of these studies (3 7) conclusions about placental transfer were based on measurements of RPBV

The use of the measurement of RPBV in the present and prior (3 7) studies as an indication of placental transfusion is justified on the basis of the data reported by Yao et al (10) who observed that when blood was transferred from the placenta to the fetus the total fetal plus placental volume remained constant The validity of the study of RPBV as an indication of placental transfusion has been discussed elsewhere (3)

In the present study a clear relationship between pH and RPBV was seen for infants of multiparous mothers thus confirming the previously suspected relationship between fetal acidosis and reduced RPBV (3) The probable physiological mechanism of placental transfer in response to acidosis will be considered below

In the present study the pH and RPBV were both slightly lower for infants of primiparous mothers (IPM s) than for infants of multiparous mothers A slightly lower RPBV would be expected for IPM s since the pH was slightly lower for these infants However the association between pH and RPBV was not clearly manifested among the IPM s even though the lowest pH values were associated with reduced values for RPBV It is possible that in some of the IPM s (see Fig 2) during the longer second stage of labor experienced by this group a decline in pH occurred only near the end of the second stage and was of too short a duration to cause a change in RPBV Additional studies utilizing sampling of fetal scalp blood are being carried out in order to determine the effect of duration of acidosis on RPBV

When large series of infants have been studied (2) a significant relationship does exist between fetal acidosis and clinical evidence of distress at birth However exceptions both in the sense of clinical distress without acidosis and acidosis without clinical distress do occur In the present series the number of infants with acidosis is too small to permit analysis of the relationship of acidosis to reduced RPBV with and without concomitant clinical evidence of distress The present data do suggest that in the infant with acidosis if there is a change in the blood volume of the infant it will be in the direction of transfer from placenta to fetus resulting in an increased neonatal blood volume While there is a clear trend only for the multiparous deliveries it is noteworthy that for primiparous deliveries *as well* there is no evidence that acidosis is ever associated with increased RPBV There is no suggestion

Table 1 *Clinical status and RPBV for infants of multiparous mothers (IMM s) and infants of primiparous mothers (IPM s) whose umbilical artery pH was more than 1 SD below their respective group mean*

pH	Apgar score 1 min	Apgar score 5 min	RPBV (ml/kg)
IMM s (mean pH 7.38)			
7.07	8	10	32
7.12	8	9	25
7.16	8	9	31
7.17	9	10	25
7.20	5	7	32
IPM s (mean pH 7.32)			
6.96	4	7	25
6.99	2	4	12
7.04	2	5	23
7.10	7	9	44

For multiparous deliveries acidosis was significantly related to reduced RPBV ($r = 0.3177$ $p < 0.05$) For example of 5 infants with pH values of 7.07–7.20 none had an RPBV value above 35 ml/kg On the other hand of 9 infants with pH values of 7.36–7.47 6 had RPBV values of above 40 ml/kg including two values of 65 and 85 ml/kg

For primiparous deliveries the relationship between pH and RPBV for the entire group was not significant However the 2 infants with pH values of less than 7.00 had small RPBV values of 12 and 25 ml/kg Very small RPBV values (below 20 ml/kg) occurred only with pH values of 7.20 or lower

DISCUSSION

This study was based on the hypothesis that placental transfusion can occur *in utero* prior to or during labor as well as after delivery Usher et al (9) investigating the blood volume of the normal full term infant found that, with immediate clamping of the umbilical cord the infant s blood volume at approximately 30 minutes after birth was 78 ml/kg but, with a delay of 5 minutes prior to clamping, the blood volume rose to 99 ml/kg Similarly Yao et al (10) found that the blood volume of the normal newborn infant *in utero* was 64% of the total fetal plus placental volume but that a rapid and step wise transfer of blood from placenta to infant occurred after delivery coinciding with uterine contractions in the third stage of labor This redistribution occurred over approximately 3 minutes at the end of which time the infant s portion of the blood volume had risen to and stabilized at 87 of the total volume

On the other hand, Yao et al (11) found that in Caesarean section fetal distress or evidence of asphyxia seemed to result in a transfer of blood, from placenta to fetus *in utero* Their value for blood volume in infants following elective Caesarean section 66 ml/kg resembled the value reported by Usher et al (9) for infants with immediate clamping while their value following asphyxial deliveries, 90 ml/kg resembled the value reported by Usher

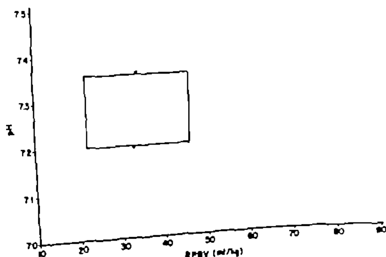


Fig 1 Umbilical artery blood pH and residual placental blood volume (RPBV) Multiparous deliveries The rectangular area includes all values within 1 SD of the mean for both pH and RPBV Acidosis was significantly related to reduced RPBV ($r = 0.3177$ $p < 0.05$)

sults of an earlier study suggested that perinatal acidosis was associated with reduced RPBV The present study confirms this association for IMM s The reason for the lack of a clearcut effect in IPM s is unknown

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Key words Perinatal acidosis residual placental blood volume placental transfusion neonatal blood volume newborn infant

Table 2 *Neonatal blood volume in various circumstances*

Circumstances at birth	Reported blood volume (ml/kg)	Reference
1 Vaginal Immediate Clamping	78	Usher et al (9)
2 Vaginal, Delayed Clamping	99	Usher et al (9)
3 Caesarean section Vigorous infant	66	Yao et al (11)
4 Caesarean section Distressed infant	90	Yao et al (11)

Blood volume is smaller for vigorous Caesarean section infants than for vigorous vaginally born infants This observation suggests that some placental transfer ordinarily occurs during labor

then that pooling of blood in the placenta and hypovolemia are likely to be a consequence of fetal acidosis

Two precautions are necessary in the interpretation of these data In most instances, redistribution of blood volume is probably due to active constriction, or dilatation of fetal or umbilical vessels In some instances however redistribution may be caused by passive compression of umbilical vessels due to extrinsic forces

Regarding active vasoconstriction Moinian et al (4) showed that the umbilical artery is more responsive to asphyxial stimuli than is the umbilical vein Therefore the expected response in acidosis would be a transfer of blood from placenta to fetus mediated by active constriction of the umbilical arteries

On the other hand in situations such as nuchal cord, passive compression of umbilical vessels may sometimes be more significant than active constriction, depending on the duration and severity of both the compression and the asphyxial changes In considering the probable effect of extrinsic forces on the umbilical vessels, the relative pressures in artery and vein become important Nyberg & Westin (5) found that umbilical artery pressure before birth of the placenta is 88/54 mmHg The same authors (6) found that the mean umbilical vein pressure is only in the vicinity of 25–30 mmHg and they also found that the venous pressure is very sensitive to the effect of uterine contractions These differences in pressure suggest that if partial compression of the cord did occur the umbilical venous circulation could be more severely affected than the arterial Consequently it is at least possible that when acidosis is associated with nuchal cord there might be pooling of blood in the placenta and reduced volume in the fetus

Likewise in Caesarean section birth the situation with respect to blood volume distribution may be complex Yao et al (11) showed that hypervolemia occurs in infants born by Caesarean section when fetal distress has occurred It also appears that some placental transfusion does occur during normal labor or in other words if neither labor nor fetal distress has occurred the blood volume of the infant is less than after normal vaginal birth (see Table 2 compare lines 1 and 3) It is unknown how long an interval must elapse before the forces involved either in normal labor or in fetal distress will cause a redistribution of blood volume Occasionally during an elective Caesarean section, the fetal pH might fall from a normal to a reduced value only very near the end of the operative procedure In such instances it seems possible that acidosis at birth might be associated with a large RPBV and hypovolemia in the infant

Our own data, summarized elsewhere, indicate that significantly larger RPBV values occur following elective Caesarean section birth and in births complicated by nuchal cord (1)

SUMMARY

Umbilical artery pH and residual placental blood volume (RPBV) were determined for 75 full term newborn infants born by vaginal delivery For 39 infants of multiparous mothers (IMM) there was a significant relationship between fetal acidosis and reduced RPBV For 36 infants of primiparous mothers (IPM) a significant relationship was not present Re

having had symptoms from childhood In a review at the same time Yater et al (36) record presenting symptoms in childhood in 25 out of 45 cases This observation is confirmed by Stringer (33) and Weiss & Gasul (34) and again by Bosher et al (6) in 1959 where 252 cases were collected from the literature and supplemented by 98 reported in reply to questionnaires The analysis of these 350 patients by Bosher also shows that in 64 of the cases it was possible to establish the presence of only a single arteriovenous fistula —most commonly in the lower lobes without any difference in side incidence

Among the remaining cases with 2 or more fistulas 23 were characterised as "multiple without any further specification Of all the cases in this group half were found to have unilateral localization and the remainder bilateral localization

No definite difference in frequency between the sexes was found in any of the studies quoted

Pathological findings

The complicated anatomic structure of the lungs with a double vascular system favours the development of vascular anomalies particularly with shunts between the arterial and venous sections of the vascular system In anatomical studies including those of von Hayek (12) it has been shown that from a physiological point of view the lungs possess arteriovenous anastomoses bypassing the alveolar capillary net Thus in normal lungs subpleural collaterals are found in the form of giant capillaries direct anastomoses between intralobular branches of the pulmonary artery and interlobular branches of the pulmonary vein and anastomoses between intralobular branches of the pulmonary artery and the bronchial venous network The blood volume thus shunted is very small and has no physiological significance apart from contributing to the fact that the blood is not quite completely saturated with oxygen after passage through the lungs

Theoretically the pathological intrapulmonary AVF could be conceived of as developing as a result of an abnormal load on the normally occurring anastomoses However such a pathogenesis is unlikely since the affected vascular regions show a pathological vascular structure and since in $^{1}/_{3}$–$^{1}/$ of the patients (10 22) there are co existing vascular anomalies in other organs skin and mucous membranes as in Rendu Osler disease The condition is in fact a congenital developmental defect which may not be symptomatic until later in life following a progressive extension of the affected vascular regions with increasing shunt formation (6 10 11 17) An attempt has been made by Anabtawi & Ellison (2) to explain the embryological mechanism for the formation of both fistulas and other known malformations of the pulmonary vessels

The macroscopic picture in AVF is exceedingly variable as appears from the review studies mentioned Most frequently a localized lesion of the lung is found in the form of a bluish swelling localized most often subpleurally with clearly visible arterial and venous vessels running into the centre where they become pronouncedly ectatic and form communicating cavities which may be pulsating There are frequently several smaller shunts with a more discrete appearance showing all transitional forms to the telangiectatic form described in particular by Hales (11) as numerous small round subpleural vascular pictures 2–5 mm in size connecting dilated peripheral branches of the pulmonary artery and vein and scattered over the surface of both lungs

Histological studies (6 9 10 11 16) show pronounced changes in the structure of the vessels Larger lesions may resemble cavernous haemangiomas (11) Changes are found in the structure of the vessel walls so that all transitional forms between arterial and venous structure occur within the same vessel wall The wall is often thickened by subintimal fibrosis or extremely thin due to loss of muscle and elastic tissue in the media Slight infiltration

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REVIEW ARTICLE

PULMONARY ARTERIOVENOUS FISTULAS IN CHILDREN

A Review with Special Reference to the Disperse Telangiectatic Type Illustrated by Report of a Case

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Pathological communication between the arterial and venous vessels in the lung may occur in three forms First in the form of one—or of a few—isolated arteriovenous fistulas (AVF) Secondly a form with several fistulas may occur (often designated multiple AVF) and finally cases have been described with innumerable telangiectatic anastomoses widespread in both lungs This last form—described in what follows as the disperse telangiectatic type (DTT)—is difficult to delimit from the multiple" form in a number of cases in the literature as the term multiple is not specified unambiguously by the authors describing it

Transitional forms have been described (1 11 15) as well as mixed cases (4 31) of the 3 forms Designations such as arteriovenous varices, arteriovenous aneurisms pulmonary angiomatosis or angiodysplasia, must be regarded as synonyms for the terms employed in the present study

Stringer et al (33) among other authors have given an account of the historic data The disease appears to have been first described in 1896 when Churton (8) demonstrated 7 walnut sized arteriovenous aneurisms in the lungs of a 12 year old boy who had had attacks of epistaxis and haemoptysis prior to his death It may also be mentioned that in 1918 Wilkens (35) described the autopsy findings in a 23 year old woman where 4 arteriovenous fistulas in the lungs during the years prior to her death had given rise to problems of roentgenological differential diagnosis from a simultaneous healed tuberculosis

It was not until the years 1949–50 however that the topic was treated in actual review studies (6 10 20 22 34 36) of both children and adults the study by Giampalmo being the most comprehensive

The disperse telangiectatic type presents the greatest diagnostic challenge, particularly where there is a problem of differential diagnosis from other pulmonary diseases and congenital heart disease Further it might be expected that the known relationship between pulmonary arteriovenous fistulas and hereditary telangiectasia (Rendu Osler disease) would here present its most striking picture

Incidence and occurrence

The incidence of AVF is elucidated by Sloan & Cooley (31) who state that 15 000 consecutive autopsies (children + adults) at The Johns Hopkins Hospital revealed only 3 cases However like other authors they stress that many cases have undoubtedly been overlooked

The review from *1950* by *Giampalmo (10)* comprises 57 cases in adults and children 29

Table 1 *Arteriovenous pulmonary fistulas in children disperse telangiectatic type*

Table no	Authors (references)	Age in years/sex	In family (visible telangiectasia and/or recurrent epistaxis)	In patient	Cyanosis	Neurological signs	Oxygen saturation of arterial blood	Roentgenogram of thorax	Cardiac catheterization/angiocardiography	Biopsy/explorative thoracotomy	Treatment	Autopsy
1	Jaffé 1929 (16)	3/4 F	−	+	0	+	0	0	0 0	0 0	0	+
	Snyder & Doan 1944 (32)	1/4 F	+	+	(+)	(+)	0	0	0 0	0 0	0	+
3	Behrend & Baer 1950 (4)	20 M	+	+	+	+	53	+	0/−	0/+	+	0
4	Brink 1950 (7)	19 F	+	+	+	−	71	+	−/−	0 0	0	0
5	Rothbauer & Blanc 1950 (25)	2 M	0	0	+	(+)	0	0	0 0	0/0	0	+
6	Szutrely & Erdelyi 1951 in Wenz & Glaml (34)	5 F	+	+	+	−	0	(+)	0/(+)	0 0	0	+
7	Mechstroth 1953 in Brunger et al (33)	15 M	0	+	+	+	0	+	0/0	0/+	+	0
8	Sloan & Cooley 1953 (31)	8 F	+	−	+	−	77	+	−/0	0 +	+	0
9	Cooley & McNamara 1954 (J)	7 F	+	+	+	−	0	(+)	0/+	+/+	0	0
10	Secrez & Fontana 1954 (27)	12 F	−	−	+	−	0	(+)	0/+	0 0	0	0
11	Hales 1955 (11)	17 M	−	+	+	+	0	0	0 0	0 0	0	+
1	Apthorp & Bates 1956 (3)	18 M	0	+	+	−	81	−	−/+	0/0	0	0
13	Husson 1956 (15)	2½ M	0	−	+	−	74	+	0/+	0/+	+	0
14	Rydell & Hoffbauer 1956 (26)	11 M	−	(+)	+	−	73	+	−/−	−/−	0	+
15	Moyer et al 1962 (22)	19 M	0	+	+	+	58	−	0/−	0/+	0	0
16	Sanders & Martt 1962 (28)	16 F	−	(+)	+	−	90	(+)	+/+	0 0	0	0
17	Kravath et al 1971 (19)	10 F	−	(+)	+	+	67	+	−/0	0 0	0	0
18	Utson & Brandrup 1972	1/2 F	−	−	+	+	54	−	−/−	0/0	0	+

+ = pos findings (+) uncertain findings − = neg findings 0 = not stated/not examined

tion As will be discussed later actual pulmonary function studies in children have been very scanty but a number of examples have been reported of cardiac catheterization and angiocardiography where the latter procedure in particular has been extremely valuable in the case of large arteriovenous pulmonary fistulas (6 17 29) but considerably more uncertain and controversial in the disperse form Table 1 includes examples of explorative thoracotomy and biopsy of lung tissue

There have been no reports of systematically controlled tests with drug or radiation therapy There have been relatively few attempts at operative treatment of children For example Jeresaty et al (17) report 2 cases of their own and collect 34 from the literature It is in the nature of things that the type of AVF which has been subjected to operative intervention is the more isolated and numerically restricted type and here the results have been satisfactory but a more detailed assessment of the results is not possible on the information available There does not appear to have been any attempt at surgical treatment in the case of recognized telangiectatic types but the report by Behrend & Baer (Table 1 No 3) is an example of the difficulties which the surgeon may encounter in cases which presumably must be interpreted as transitional forms between multiple and telangiectatic fistulas

The following case report illustrates several characteristic features and in Table 1 a schematic survey is given of cases collected from the literature which can be referred either to "pure" or to mainly telangiectatic types

with lymphocytes and macrophages is often seen around the abnormal vessels In extreme cases perifocal sclerosis and atelectasis are found in the surrounding lung tissue

Pathophysiology

The pathophysiology of the condition is an intrapulmonary right–left shunt, resulting in varying degrees of reduced O saturation in the systemic circulation The effect of several minor shunts is in principle the same as that of one or a few large shunts but as will be discussed later it is reasonable in a case of disperse telangiectatic fistulas to assume that a considerable variability in the degree of shunting is characteristic and that the result of breathing pure oxygen may vary Diffusion in the lung is not primarily affected nor is there primarily any cardiac effect of haemodynamic significance even though in a case of solitary arteriovenous fistula it is possible to find abnormal pressure/flow values in the heart lung circulation during exercise as described by Hultgren & Gerbode (14)

Clinical symptoms and genetic aspects

In agreement with the pathophysiological findings the primary clinical symptoms are cyanosis and dyspnoea Digital clubbing is often seen and the reduced arterial oxygen saturation is accompanied by polycythaemia Neurological symptoms are pronounced Presumably several factors are operating—hypoxaemia, polycythaemia increased risk of thrombosis/embolism and possibly at the same time intracranial vascular malformations The symptoms are correspondingly variegated headache dizziness tinnitus, paraesthesias and possibly syncope and convulsions

Finally the incidence of cerebral abscess is clearly increased Thus among other reports, Stringer et al (33) in their review mention 5 cases among 140 patients Cases with haemoptysis occur regularly and haemothorax has been described as a sequel to ruptured AV fistula

The familial occurrence of AVF has been reported repeatedly and there is much to suggest the presence in these cases of an obligatory relationship with hereditary telangiectasia (Rendu Osler) whether this is manifest clinically in the patients or is shown to be probable from genetic investigation (5 20 23) In the reviews quoted (children + adults) telangiectases are found in the skin/mucous membrane in 1/3 to 1/2 of the patients Moyer et al (22) however find telangiectases in 2/3 of 21 patients In their analysis of a family with Rendu Osler disease, Hodgson et al (13) found 14 cases of AVF among those 91 members of the family who had recognized telangiectases but none among the other 140 members Rendu Osler disease is considered a hereditary disease with autosomal dominance, but possessing a rather varying degree of manifestation and with cases of apparent atavism (32)

Diagnosis and treatment

Asymptomatic cases of arteriovenous fistulas are not uncommonly revealed as the result of a routine roentgenological examination of the thorax (22) In the above mentioned investigation of a Rendu Osler family by Hodgson, 8 cases of asymptomatic arteriovenous pulmonary fistulas were found in 125 routine roentgenograms of the thorax

Because of the cyanosis and the possible murmur from the fistula on stethoscopy of the thorax congenital heart disease will be suspected in most cases of symptomatic AVF In the isolated forms, the roentgenograms of the thorax often shows tumour like shadows On tomography and in particular on fluoroscopy during the Valsalva and the Muller manoeuvre whereby the possibly pulsating tumour is alternately reduced or increased in size, a very great degree of diagnostic certainty may be achieved In multiple and disperse forms increased vascular markings are often described in the lungs

In recent years most of the patients in the cases reported have undergone extensive investigations of the heart and pulmonary func



Fig. 1. Left lung. Stasis in the subpleural veins in the interlobular space. Numerous dark spots each representing a telangiectasis up to 3 mm in diameter.

but normal temperature. As previously, no murmur on ste hoscopy of the thorax, but roentgenogram of the thorax showed cardiac enlargement and bilateral markings of stasis in the lungs. No skin changes observed.

Lumbar puncture and spinal fluid examination (including culture) showed nothing abnormal. The patient's condition rapidly deteriorated with severe anoxia and intractable progressive failure of the heart and lungs, and death ensued on the same day.

Autopsy

Lungs were normal in size with glistening, smooth surfaces. Colour varied somewhat, marbled with subpleural bluish-red regions. All over both lungs uniformly distributed were innumerable subpleural telangiectases with central vessels up to 3 mm in diameter continuing in exceedingly tangled hair-fine darker vessels (Fig. 1). Interlobularly pronounced stasis in the subpleural veins. Normal morphology of the central pulmonary vessels.

Heart moderately enlarged. Right atrium slightly dilated but not hypertrophic. Ventricles normal. Heart and its vessels showed normal morphology. In particular the ductus arteriosus and the foramen ovale were closed. No septal defects found, heart valves normal. Pulmonary veins opened into the left atrium in a normal manner.

Normal conditions were found in the other organs, in particular no telangiectases on the mucous membranes or in other organs than the lungs. No sign of extrapulmonary vascular anomalies, in particular the cerebral vessels showed no changes.

Histological examination of tissue from the mastoid region and from the palm of the hand showed normally structured skin without telangiectases.

Histological examination of the lung tissue in particular subpleurally showed numerous large and small dilated vessels, in places with a suggestion of emphysema and atelectasis of the surrounding lung tissue. No inflammatory infiltration (Fig. 2). Elastin staining demonstrated dilated vessels where the elastic fibres in the lamina media varied between arterial and venous construction in the same vessel wall, such as seen in arteriovenous shunts (Fig. 3).

In addition, the left lung was dissected free and barium sulphate suspension injected through a catheter to the pulmonary artery while performing fluoroscopy simultaneously. The contrast penetrated rapidly into a somewhat dilated vascular system, and shortly after commencing the injection contrast was seen right out subpleurally in the fine twisted vessels corresponding to the telangiectases found at autopsy (Fig. 4).

Roentgenogram of the lungs showed dilated plexuses of vessels at several peripheral sites in the lungs, suggesting arteriovenous anastomoses (Fig. 5).

DISCUSSION

The presentation in Table 1 of previously reported cases together with the present case permits an evaluation of whether it is reasonable to distinguish the disperse telangiectatic type as a clinical subgroup. We found it important to elucidate the relationship to Rendu-Osler disease, as it was considered reasonable to assume that multiple and particularly telangiectatic types of pulmonary fistulas might confirm the occurrence of a common denominator between these and Rendu-Osler disease, which must be regarded as generalized angio-dysplasia. Several authors have been tempted to equate the two diseases, but have based their observations on both children and adults, and on both isolated and multiple fistulas.

Among the cases in Table 1 we find presumably or possibly co-existing Rendu-Osler disease in 14 out of the 18 patients. This is a

Table 2 *Laboratory findings on arterial puncture before and after oxygen inhalation*

Date		Po_2 (mmHg)	O_2 sat ( )	Pco_2 (mmHg)	pH	St bicarb (mEq/l)	Hb (g/100 ml)	Eryth (mill/μl)	Haematocrite (ml/100 ml)
	Atmospheric air								
23/2 1971	Patient at rest	34	54	29	7 29	14 5	15 5	5 8	58
	Couveuse 30 min O_2 saturation 90–95	44	72	24	7 32	12 0			
18/3 1971	Universal anaesthesia 15 min O_2 5 l/min Fluothane 0 5	52	80	32	7 31	15 0	16 6	5 9	61
26/3 1971							18 0	7 1	61

CASE REPORT

1st admission Three month old girl admitted as an emergency in February 1971 for suspected heart disease The first and only infant of healthy parents Pregnancy and delivery uneventful Birth weight 2 550 g and length 51 cm From birth slight generalized cyanosis noticed particularly peripherally on the extremities but also on the body and even when the child was at rest

The infant s condition was unremarkable and she was normally developed There was slight cyanosis of the body pronounced acrocyanosis and moderate tachypnea No murmurs on stethoscopy of the thorax no hepatomegaly General physical examination other wise unremarkable ECG and roentgenogram of the thorax normal Angiocardiography and cardiac catheterization were performed via the right femoral vein The catheter was led through the foramen ovale and contrast fluid was injected into the left atrium the right atrium and the pulmonary artery Morphological picture was completely normal—in particular no evidence of pulmonary or intracardiac shunts

Simultaneous pressure measurements in mmHg showed the following valu s pulmonary artery 13/3 right ventricle low 16/−2 middle of right atrium 1 middle of left atrium 6 left ventricle 60/−7 These values are likewise normal

Oxygen saturation percentages were right pulmonary artery 49 central pulmonary artery 51 right ventricular conus 49 bas of right ventricle 48 superior vena cava 46 right atrium 54 inferior vena cava 60 pulmonary vein 80 left atrium 76 left ventricle 72 Low oxygen saturation was thus found throughout also in the pulmonary veins

Blood examination during th same period showed total haemoglobin concentration 18 6 g/100 ml and erythrocyte volume 60 Methaemoglobin 2 sulphaemoglobin 0 CO haemoglobin 0 5 haemo globin-oxygen capacity normal Haemoglobin-electrophoresis normal 12 2 of haemoglobin alkali resistant

Table 2 shows the result of arterial puncture When incubation was tried the cyanosis disappeared but it was present during the actual puncture wh n th infant was agitated

Other routine laboratory tests all showed normal conditions The infant was discharged to outpatient follow up with th aim of supplementary investigations later

2nd admission One week later the infant was re admitted as an emergency after fever and agitation for 1 day On admission febrile exhausted irritable screaming with rapid respiration and moderate general cyanosis

Lumbar puncture showed a clear spinal fluid with pleocytosis As a result of treatment with antibiotics (penicillin sulpha streptomycin) the temperature was rapidly normalized and her general condition improved Blood culture and spinal fluid culture were negative Spinal fluid protein 116 mg/100 ml spinal fluid glucose 43 mg/100 ml Cell count of spinal fluid 4 200 leucocytes μl with 78 polynuclear and 12 mononuclear Erythrocytes 0

Ro ntgenograms of th thorax showed increased vascular markings without any cardiac enlargement

ECG nothing abnormal

After treatment for 1 week the patient s condition was as usual with generalized cyanosis and in addition incipi nt clubbing of fingers and toes As diffuse intrapulmonary right–left shunts were suspected arterial puncture was performed during this admission after administering pure oxygen Results are shown in Table 2 During outpatient observation over the next 2 months a progression was seen in her condition with slightly increasing cyanosis tachypnea tachycardia and clubbing The patient s polycythaemia likewise increased (Table 2) Her general condition however remained good and psychomotor d velopment was normal

3rd admission Re admitted 6 months old after 3 days with affected general condition increasing cyanosis and tachypnea On hospitalization stuporous with severe cyanosis and rapid respiration and pulse



Fig 4 Left lung Shortly after commencing the injection of contrast Subpleural telangiectatic vessel

verify numerically that the total amount of blood shunted through the lungs has increased but as mentioned previously the pathological picture presents evidence for assuming that the abnormal arteriovenous communications are not stationary but can be redistributed and increased during tissue breakdown

The meningitis like condition in our patient on admission to hospital on the second occasion agrees with the frequent occurrence of cerebral signs previously mentioned including brain abscess and is remarkably similar to the case reported by Hales (Table 1 No 11) The picture presented by our patient at death may be interpreted as hypoxic cerebral and cardiac damage

The results of the technically satisfactory cardiac catheterization and angiocardiography which were carried out in our patient refute the suspicion of congenital heart disease As is seen from the summarized case histories in Table 1 angiocardiography has in a number of cases provided evidence supporting the diagnosis of DTT (6 9 10 12 Table 1) In the present case a review of the video-tape recordings of the cineangiocardiography gave rise to a suspicion of an abnormal peripheral vascular picture and we consider it probable that the diagnosis could have been verified *in vivo* if there had been a possibility of a repeated angiocardiography with special attention to the peripheral pulmonary vessels—preferably in the form of plate angiocardiography which provides great picture resolution

Also in case 16 Table 1 angiocardiography gave rise to a suspicion of arteriovenous pulmonary fistulas and catheterization of the pulmonary vein through the atrial septal defect presumably gave a unique opportunity to sub-



Fig 5 Left lung Roentgenogram after injection of contrast into pulmonary artery Peripherally particularly apically pronounced dilated vascular plexuses are seen



Fig 2 Left lung Increased vascular density Numerous large and small dilated vessels Emphysema and atelectasis of surrounding pulmonary tissue H E ×57



Fig 3 Left lung Centrally large transversely cut vessel Elastic fibres in the lamina media vary between arterial and venous structure Elastin staining ×57

higher figure than found in any report previously quoted but the heterogenous nature of the material does not permit any far reaching conclusions However it should be pointed out that this is a material of children and young people and that it is well known that Rendu Osler disease may not become manifest until the second or even the third decade In addition the milder forms of the disease may easily be misinterpreted In contrast case No 2 in Table 1 may be emphasized as apparently representing the (lethal ?) homozygous form of the disease In our own case telangiectasis was not demonstrated either on inspection of the skin or mucous membranes or as a result of skin biopsies at autopsy The parents of the patients were examined in the dermatological department of the hospital with a view to finding possible telangiectases and in the roentgen department with a view to possible pulmonary fistulas Both examinations were negative No cases of disease compatible with Rendu Osler disease are known in the kinship The present case therefore cannot be taken as evidence of an obligatory relationship between Rendu Osler disease and arteriovenous pulmonary fistulas not even in the telangiectatic type of the latter

In most of the patients reported cyanosis presented later than in the present patient where the disease also showed a surprisingly rapid clinical progress It is not possible to

disease telangiectasia hereditaria (Rendu Osler disease) is discussed It is emphasized that this relationship appears particularly clear in the telangiectatic type a circumstance which is however not confirmed in the present case The difficulties in diagnosis and the diagnostic procedures are mentioned

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stantiate the suspicion by determinations of oxygen tension as previously mentioned In our patient the absence of changes in haemoglobin and the absence of an intracardiac shunt the low oxygen tension in the systemic circulation and the P_{CO} which was normal at the same time all pointed strongly in the direction of extra cardiac shunts the more so as the normal roentgenogram of the lungs at this stage made a massively reduced diffusion capacity unlikely Trials of breathing pure oxygen (Table 2) must then be expected to result in only a slight rise and as seen a P_O of 52 was found corresponding to a right–left shunt of about 60° (18)

The patient of Apthorp & Bates (Table 1 No 12) who was only slightly desaturated was found to reach almost complete saturation when breathing 100 oxygen Le Roux (20) and Kravath et al (Table 1 No 17) found similar conditions in their patients This may presumably be explained by the dilatation of the lung vessels caused by breathing oxygen (24) The result is a haemodynamic redistribution which alters the total effect of the pathological shunts Apthorp & Bates (3) also suggest that the telangiectatic shunts are so near the alveoli that a high O_2 tension here will be able to produce oxygenation of the shunt blood

This is in agreement with the clinical effect of oxygen breathing in our patient, as the habitual obvious cyanosis practically disappeared during the actual trial

Our assumption that arteriovenous pulmonary fistulas are often overlooked—even at autopsy—was strengthened after the death of our patient where the pronounced picture of dark red telangiectatic foci subpleurally had almost disappeared a few hours after the organs were removed As mentioned, Hales (11) and Rydell & Hoffbauer (26) have reported attempts to produce vinyl casts of the vessels in connection with autopsy In the first case the solution appears to have been too viscous to give a completely satisfactory result while in the second case subpleural shunts less than 1 mm in size could be demonstrated but an exact evaluation of the size was difficult on account of the shrinkage of the plastic mass The injection pressure was not recorded The demonstration of the vascular tree by the injection of contrast medium accompanied by roentgenograms has been reported by Sloan & Cooley (31) and Silverman et al (30) and our technique is in line with theirs The advantage of this method is that it can rapidly be put into practice and is easy to improvise In our case we consider that the results with this technique have been decisive for verifying the diagnosis and we feel that it can be recommended in similar cases in future

Our patient showed no sign of haemorrhagic diathesis and the thrombocyte state was not investigated 2 patients (Table 1 Nos 12 and 17) underwent splenectomy on account of thrombocytopenia We also found a normal liver in our patient at autopsy which is of interest, as a relationship between arteriovenous pulmonary fistulas and liver disease has been pointed out by several authors (34 30) and in fact also confirmed in the present review where in cases Nos 14 and 17 Table 1 liver biopsy showed cirrhosis while the biopsy in case No 12 showed haemangiomas in the liver

SUMMARY

Arteriovenous pulmonary fistulas present in infancy in more than half the number of cases A distinction is drawn between isolated and multiple fistulas and as a special category of the latter the disperse telangiectatic type, is described

A review of the literature reveals 17 cases of this type The present case extends this series—a girl dying at the age of 6 months, in whom the diagnosis of disperse telangiectatic arteriovenous pulmonary fistulas was regarded as probable on the basis of the investigations made The diagnosis was confirmed at autopsy which included bronchovascular injection of contrast during fluoroscopy

The relationship to the dominant hereditary

disease telangiectasia hereditaria (Rendu-Osler disease) is discussed It is emphasized that this relationship appears particularly clear in the telangiectatic type a circumstance which is however not confirmed in the present case The difficulties in diagnosis and the diagnostic procedures are mentioned

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Pathologist J Ibsen has contributed considerably by her interest in the histological diagnoses

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Key words Pulmonary arteriovenous fistulas dispers telangiectatic type telangiectasia hereditaria (Rendu Osler) children

APPENDIX

Comments to Table 1

No 3 Cyanosis for 3 years Treated for poly cythaemia Angiocardiography unsuccessful Thor acotomy performed 3 times Right and left lower lobes of the lungs removed together with numerous 0 5–2 0 cm large haemangiomas (+abnormal vessels through the right diaphragm) The other lobes appeared nor mal but the left upper lobe has since been removed together with a large AV fistula corresponding to the whole lingula Thereafter no clinical cyanosis—but only 9? 8 arterial O_2-saturation on inhalation of 100 O_2

No 9 Cyanosis for 6 months Roentgenogram of thorax pronounced peripheral markings Angiocardio graphy curious beaded appearance extending out in the peripheral pulmonary *zones Explorative thor* acotomy at least 15 telangiectatic areas on the sur face Biopsy of one of the largest (3 mm) showed "angiomatous process which appeared to be *of* a mature type"

No 11 Cyanosis from the age of 10 years Epistax and lipothymia Died in a state resembling purulen meningitis (Negative spinal fluid culture) Autopsy n malformations in the heart or in the large vessel Several 2–5 mm large dense plexuses of tiny vessels subpleurally in both lungs Bronchovascular cast (vin lite+diatomaceous earth) shows several hundred AV *fistulas—also deeper in the lung tissue*

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With a catheter in the pulmonary vein O_2 satura tion of 76 was obtained but 100 in the pulmonar artery left distal Wedge Diffusing capacity (carbo *monoxide single breath method) normal*

Acta Pædiat Scand 62 433–436 1973

SHORT COMMUNICATION

CONTINUOUS POSITIVE AIRWAY PRESSURE WITH A FACE CHAMBER IN EARLY TREATMENT OF IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

HANS AHLSTRÖM BJÖRN JONSON and NILS W SVENNINGSEN

From the Departments of Pædiatrics and Clinical Physiology University of Lund Lund Sweden

A decreasing mortality rate in infants with idiopathic respiratory distress syndrome (IRDS) has been reported after the introduction of treatment with continuous positive airway pressure (CPAP) (1 4 5 11) A new equipment for CPAP treatment without intubation is described below together with preliminary results

METHODS

On e g an infant s intensive care crib (3) a face chamber is mounted The infant s face protrudes into the face chamber through a diaphragm (Fig 1) a disposable latex ring filled with styrene particles Four different sizes are needed It is lax and easily adapted to fit tightly around the face To maintain tightness the air is evacuated from the interior of the ring which then becomes inflexible The lid of the chamber is then attached It can quickly be removed Air and oxygen is led via a bacteria proof filter and an air conditioner (35 C saturated with water) to the face chamber The flow rate was 1–15 l min enough to eliminate the dead space effect of the chamber At the outlet of the chamber the gas passes a pressure regulating valve that can be adjusted to produce pressures up to 15 cm H_2O The pressure is monitored on a manometer The face chamber is connected to safety waterseal which opens at 15 cm H_2O (An equipment according to the principles above will be available from Siemens-Elema AB Solna Sweden)

PROCEDURES

From November 1972 to March 1973 the diagnosis IRDS was recognized in 14 infants in the neonatal unit in Lund (Table 1) We have used the following *diagnostic criteria* of IRDS *Clinical* Within 8 hours of age grunting tachypnoea (above 60 per minute) intercostal retractions and cyanosis *Laboratory* Hypoxia as described below and arterial pH below 7 2 Pulmonary X ray showing typical reticulo-granular pattern was considered supportive While the infant was breathing 40% O arterial blood from an umbilical catheter was analysed for partial pressure of O_2 and CO_2 (Pa_{O_2} and Pa_{CO_2}) and acid base data After breathing 100% O for 10 min a new analysis of Pa_{O_2} was made (*hyperoxia test*) If this Pa_{O_2} was less than 100 mmHg IRDS was diagnosed if not the hyperoxia test was repeated every 2 to 6 hours during the first day of life for reevaluation CPAP treatment was started as soon as the criteria were fulfilled in ten cases Conventional therapy was given in four cases A hyperoxia test was performed within one hour after the start of CPAP The oxygen concentration was adjusted so as to maintain a Pa_{O_2} of 50–90 mmHg

CPAP treatment was terminated when grunting and cyanosis did not reappear after lowering the pressure to zero and when the acid base and Pa_{O_2} values 30 to 60 min later were satisfactory

RESULTS

Out of four neonates (Nos 11–14 Table 1) who were treated without CPAP three required intermittent positive pressure ventilation IPPV One of these infants (case 11) died. IPPV was complicated by pneumothorax and pneumopericardium in case 14

Ten neonates (Nos 1–10 Tables 1) were

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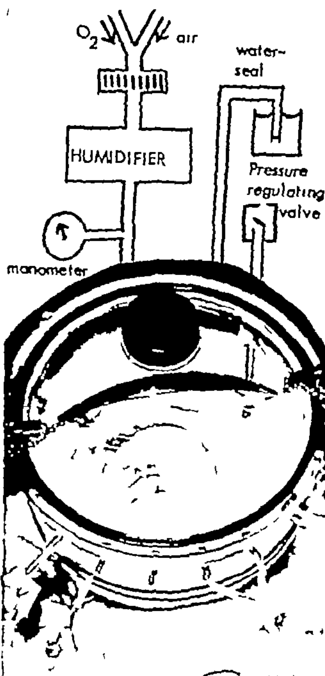


Fig. 1 Face chamber and associated equipment described in text

initially treated via the face chamber Two infants died (Nos 4 and 8, for diagnosis see Table 1)

Eight patients survived No 6 got apneic spells during CPAP treatment because of coli meningitis In the seven successfully CPAP treated infants improvement of the hyperoxia test was found within the first hour of treatment (Table 1) In all of them the grunting subsided as soon as an adequate pressure (4–12 cm H O) was applied This is a valuable sign of effective treatment

Pa_{O_2} (100 % O) before CPAP was lower than 70 mmHg in 8 infants and between 70 and 100 mmHg in 2 infants 30 min after the start of CPAP an oxygen concentration of 40% in the face chamber gave similar Pa_O values as 100 O did before (see Table 1) Besides this the hyperoxia test during CPAP showed a dramatic improvement in 8 out of 10 infants The good effect allowed the inspired oxygen concentration to be below 40% during CPAP in all but 2 infants Pa_{CO} was 55–80 mmHg before treatment but decreased to values below 50 mmHg during CPAP in all subjects except No 8

DISCUSSION

No complication referrable to CPAP was noted The treatment was found very convenient in contrast to the common experience with IPPV

Intubation in the CPAP treatment of infants involves several well known disadvantages (8 9) Furthermore grunting is a valuable clinical sign indicating a need for increased alveolar pressure (6) It is lost at intubation

Earlier described equipment for CPAP treatment without intubation (1 5) may involve risks of congestion of the veins of the neck and also risks for cochlear damage in immature babies by high noise levels (2) The face chamber allows easy nursing Thus it is favourable from several points of view to use a face chamber

The present study was started with the aim to perform a randomized comparison between CPAP and conventional treatment including IPPV when necessary The dramatic effect of CPAP observed after a brief period of treatment in all patients in which IRDS was found to be the main diagnosis forced us to abandon the randomized study

According to our experiences CPAP treatment should be started as soon as the diag

Table I *Clinical data and treatment of 14 neonates with IRDS (idiopathic respiratory distress syndrome)*
The case numbers do not refer to the time sequence of treatment (see text). GA = gestational age

Case sex	Weight (g)	GA (w)	Diagnosis	CPAP treatment: Age at start (h)	CPAP treatment: Duration (h)	Deaths (age h)	Hyperoxiatest (Pa_{O_2} mmHg): Before treatment 40 O₂-100 %	Hyperoxiatest (Pa_{O_2} mmHg): During CPAP 40 O₂-100 %
1 ♀	1 860	34	IRDS	5	13		19 65	65 195
2 ♀	1 830	34	IRDS	12	15		31 69	59 215
3 ♀	2 250	35	IRDS	5	7		38 – 67	61 168
4 ○	1 160	29	IRDS + ICH	5	23	36	42 – 52	185 – 230
5 ♀	2 370	33	IRDS + hypothyreosis	24	97		36 – 49	0 – 140
6 ♂	1 930	32	IRDS + meningitis	3	5		18 – 26	45 – 0
7 ♂	2 500	35	IRDS + pneumothorax	11	44		35 – 65	60 – 210
8 ♂	1 510	30	Postasphyxia[1]	2	5	17	20 – 40	30 – 0
9 ♂	500	34	IRDS	12	27		53 – 98	65 125
10 ♂	2 870	36	IRDS	7	31		49 – 76	6 – 220
11 ○	1 550	30	IRDS	IPPV		36	25 – 49	
12 ♂	2 900	36	IRDS	—			34 – 95	
13 ♀	1 290	29	IRDS	IPPV			35 – 70	
14 ☿	2 100	33	IRDS pneumothorax + pneumopericardium	IPPV			28 – 55	

ICH = intracranial hemorrhage (at autopsy rupture of tentorium cerebelli and intraventricular hemorrhage)
Postasphyxia syndrome severe perinatal asphyxia (at autopsy cerebral anoxia and meconiumaspiration in the lungs)
IRDS (at autopsy massive hyaline membranes in the lungs)

nous IRDS is established. A delay will imply a risk for further deterioration and ischemic intracranial damage (7, 10). We have a strong feeling that the good results of CPAP treatment are due to its *early* application and therefore assert that a liberal use of CPAP in the early treatment of IRDS will radically change the prognosis. Such a therapeutic program will necessarily involve the treatment of some infants that would survive with conventional care. This appears justified since no complications have been noticed.

In infants with IRDS a prompt effect can be expected. If the effect is slow or absent some other diagnosis must be suspected (Nos 6 and 8).

SUMMARY

CPAP treatment started early with a new face chamber was found to be convenient and without risks. Treatment of IRDS applied early with the presented technique appears to radically improve the prognosis.

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CASE REPORT

INTERVERTEBRAL DISC CALCIFICATION IN CHILDHOOD

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In childhood intervertebral disc calcification (IVDC) is a rare finding but when present is often associated with a characteristic clinical picture including focal symptoms as well as signs of systemic disease Since 1924 approximately 75 cases in childhood have been reported (1 2 3 4 5 6 7 8 9) mostly in the radiological literature A few cases have been reported from Scandinavia (2 3 9)

CASE REPORT

J R a 6 year-old boy was admitted to the pediatric department because of poor appetite headache and increasing pain in the back and neck for the previous week He had earlier been in good health

On admission he was extremely stiff in his neck and back and exhibited tenderness of the spine in the interscapular region Otherwise the physical examination was normal

X ray of the vertebral column showed calcifications in the 11th and 12th thoracic intervertebral disc (Fig 1) Bone age corresponded to a chronological age of 3 years (Greulich & Pyle) X ray of the rest of the skeleton and chest was normal

Laboratory investigations showed the sedimentation rate elevated to 46 mm/h normal hemoglobin concentration and leucocyte count Serological tests for ornithosis and influenza were negative A throat culture gave no growth of hemolytic streptococci Antistreptolysin and antistreptohyaluronidase titres were slightly elevated In the spinal fluid, cell counts and culture showed no signs of bacterial or viral infection

Serum calcium serum phosphorus, serum alkaline phosphatase and urinary calcium excretion wer all within normal limits Serum sodium, serum potassium serum creatinine and blood urea were also normal Serum proteins, serum transaminases, and serum lactic acid dehydrogenase were normal The Rose Waaler test rheumatoid arthritis test and examination for antinuclear factors, were negative In a blood smear no L.E. cells were found Urinary excretion of homogentisinic acid porphyrins and aminoacids was not increased Serum thyroxine was normal Eye examination (slit lamp) showed no signs of metastatic calcifications An electrocardiogram showed no abnormalities

The further clinical course was uneventful and the pain and rigidity of the boy s neck and back gradually disappeared For the first week his rectal temperature was slightly elevated The sedimentation rate decreased 10 days after admission he was without symptoms

Seven months after admission he is still clinically well However roentgenographically the intervertebral disc calcifications are unchanged

DISCUSSION

IVDC is equally frequent among girls and boys (5) Most often the disease occurs in late childhood but cases in the neonatal period have been reported (1 5)

Any disc in the vertebral column may be affected On roentgenograms round or oval solitary or fragmentary calcifications are seen most often in the nucleus pulposus

Characteristic symptoms may be seen in 80% of the pediatric cases of IVDC (5) These are pains and stiffness in the neck and back sometimes accompanied by torticollis general malaise with poor appetite and low grade fever In a few weeks the symptoms gradually

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Acta Pædiat Scand 62 437–439 1973

CASE REPORT

INTERVERTEBRAL DISC CALCIFICATION IN CHILDHOOD

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In childhood intervertebral disc calcification (IVDC) is a rare finding but when present is often associated with a characteristic clinical picture including focal symptoms as well as signs of systemic disease Since 1924 approximately 75 cases in childhood have been reported (1 2 3 4 5 6 7 8 9) mostly in the radiological literature A few cases have been reported from Scandinavia (2 3 9)

CASE REPORT

J R a 6 year old boy was admitted to the pediatric department because of poor appetite headache and increasing pain in the back and neck for the previous week He had earlier been in good health

On admission he was extremely stiff in his neck and back and exhibited tenderness of the spine in the interscapular region Otherwise the physical examination was normal

X ray of the vertebral column showed calcifications in the 11th and 12th thoracic intervertebral disc (Fig 1) Bone age corresponded to a chronological age of 3 years (Greulich & Pyle) X ray of the rest of the skeleton and chest was normal

Laboratory investigations showed the sedimentation rate elevated to 46 mm/h normal hemoglobin concentration and leucocyte count Serological t s s for ornithosis and influenza were negative A throat culture gave no growth of hemolytic streptococci Antistreptolysin and antistreptohyaluronidase titres were slightly elevated In the spinal fluid, cell counts and culture showed no signs of bacterial or viral infection

Serum calcium serum phosphorus serum alkaline phosphatase and urinary calcium excretion were all within normal limits Serum sodium serum potassium serum creatinine and blood urea were also normal Serum proteins, serum transaminases, and serum lactic acid dehydrogenase were normal The Rose Waaler test rheumatoid arthritis test and examination for antinuclear factors were negative In a blood smear no LE cells were found Urinary excretion of homogentisic acid porphyrins and aminoacids was not increased Serum thyroxine was normal Eye examination (slit lamp) showed no signs of metastatic calcifications An electrocardiogram showed no abnormalities

The further clinical course was uneventful and the pain and rigidity of the boy s neck and back gradually disappeared For the first week his rectal temperature was slightly elevated The sedimentation rate decreased 10 days after admission he was without symptoms

Seven months after admission he is still clinically well However roentgenographically the intervertebral disc calcifications are unchanged

DISCUSSION

IVDC is equally frequent among girls and boys (5) Most often the disease occurs in late childhood but cases in the neonatal period have been reported (1 5)

Any disc in the vertebral column may be affected On roentgenograms round or oval solitary or fragmentary calcifications are seen most often in the nucleus pulposus

Characteristic symptoms may be seen in 80 of the pediatric cases of IVDC (5) These are pains and stiffness in the neck and back sometimes accompanied by torticollis general malaise with poor appetite and low grade fever In a few weeks the symptoms gradually





Fig 1 (*a*) Frontal view (*b*) Lateral view (tomogram) Calcification in the nucl us pulposus of the 11th and 12th intervertebral thoracic discs with marked erosion of adjacent bone in the vertebral bodies

recede Roentgenographic regression of the calcifications may accompany disappearance of clinical symptoms but usually complete regression is not seen till 1 or 2 years after the acute phase The thoracic calcifications seem the most persistent

Apart from increased sedimentation rate and leucocytosis laboratory investigations have been negative Abnormalities in calcium metabolism metastatic calcifications in other tissues and evidence of vitamin D intoxica tion have never been reported Association with extravertebral bone or joint disease has not previously been noticed

The cause of the disease is obscure The fact that IVDC may be seen in neonatal period indicate that the calcifications at least in some cases have a prenatal etiology In addition several cases are known in which calcifica tions are present without clinical symptoms or before symptoms appear (5 6 10) Perhaps exogenic factors for instance *infection* or trauma bring about circulatory disturbances in the affected discs with a subsequent libera tion of calcium salts and an ensuing local in flammatory reaction This hypothesis has also been proposed by other authors (6 10)

In the reported case no explanation of the decreased bone age can be given The finding may be casual and unrelated to the observed IVDC However in cases of IVDC roentgeno-graphic examination of the entire skeleton should be performed

SUMMARY

Intervertebral disc calcification (IVDC) is a disease seldom reported in the pediatric litera ture It is often associated with local symp toms from the back and signs of systemic dis ease A recent case in a 6 year old boy is re ported

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Key words Intervertebral disc calcification children

Acta Pædiat Scand 62 440–444 1973

CASE REPORT

MALE PSEUDOHERMAPHRODITISM IN A PATIENT WITH E TRISOMY SYNDROME

G FLUGE A MYKING and D AARSKOG

From the Department of Paediatrics and the Department of Pathology The Gade Institute University of Bergen Norway

E trisomy syndrome is a well defined clinical entity including failure to thrive mental retardation hypertonia a fairly characteristic physiognomy and a multitude of congenital malformations involving most organ systems The reported anomalies affecting the genito-urinary systems include Undescended testes, small penis enlarged external genitalia in girls, horse shoe kidney, renal agenesia retention cysts hydronephrosis and hydro ureter (4) The underlying chromosome abnormality has given the syndrome its name

Male pseudohermaphroditism defined as a condition in which the genetic and gonadal sex is male but the internal or external genital organs are sufficiently ambiguous to give rise to uncertainty as to the sex does not appear to have been observed in the E trisomy syndrome The present report describes such a case

CASE REPORT

The patient was born to a 30 year old healthy woman at 38 weeks gestation and admitted to the Children's Hospital in Bergen 1 hour after delivery Two previous pregnancies had resulted in normal children The parents were not related There were no cases of congenital malformations or pseudohermaphroditism among the near relatives The pregnancy was uneventful and there was no history of viral infection radiation exposure or drug ingestion

The infant was asphyxiated at birth with an Apgar score of 2 points Hydramnion was noted The birth weight was 1 810 g and the length 42 cm Several congenital anomalies were observed malformed ears small eyes micrognathia small mouth and a high arched palate flexion deformities of the fingers, dysplastic nails short sternum limited hip abduction rocker bottom feet and short dorsiflexed halluces The penis was of normal masculine shape and size with a central glandular urethral meatus A gonad was palpated in the right inguinal canal There was a precordial systolic murmur suggestive of congenital heart disease Dermatoglyphs showed simple arches on fingertips and distal situated right palmar triradius Chromosome analysis from peripheral blood showed 47 chromosomes with an extra chromosome No 18 (47 XY 18+)

The infant made a temporary recovery after resuscitation but later his general condition deteriorated and he died when 4 days old At autopsy the findings were Hypoplasia of the olfactory bulb on both sides cardiac anomalies with a wide ventricular septal defect valvular and post valvular pulmonary stenosis a wide and slightly dextroposed aorta and persistent ductus arteriosus No anomalies were noted on examination of the gastro intestinal tract kidneys or the urinary system The adrenal glands were normal both on gross and microscopic examination The internal genitalia were ambiguous with a bicornuate uterus dorsal to the urinary bladder Rudimentary Fallopian tubes were connected to the uterine cornua (Fig 1 and 2 *a*) On cross section the Fallopian tubes showed rudimentary folding of the propria which was lined by a single layer of cylindrical cells (Fig 2 *b*) No ovaries were found The rudimentary utero vaginal canal was lined by high stratified squamous epithelium (Fig 2 *c*)

The testes and epididymis were located at the inner inguinal annulus The microscopic appearance was normal with well developed seminiferous tubules lined by stratified cuboidal-cylindrical epithelium with mainly round dense nuclei and scarce compact slightly eosinophilic cytoplasm (Sertoli cells) (Fig 3 *a*

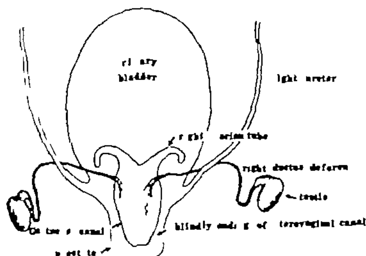


Fig. 1 Drawing showing the main pelvic organs from behind

and 3 b) Close to the basement membrane scattered large cells with clear cytoplasm (spermatogonia) were found (Fig. 3 b) Interstitial cells were well developed in the interlobular connective tissue (Fig. 3 c) The rete testis and epididymis showed a normal micro scopic appearance The deferential ducts were hypo plastic with a diameter of 0.5 mm lined by regular cylindrical epithelium surrounded by well-developed muscle layers They continued medially and ended as Gartner's canals in the muscle layers on both sides of the rudimentary [utero-vaginal] canal (Fig. 1 2 a and 2 c) The prostatic gland was normal on gross and microscopic examination

DISCUSSION

Recent advances in experimental embryology cytogenetics and steroid biochemistry have ac cumulated considerable information on the mechanism of sex determination and differen tiation These data have substantiated the clas sic concept that most sexual characteristics emerge from bivalent or indifferent precursors in the embryo and that a wide spectrum of differentiation is possible at each level of sexual organization

The normal differentiation of the gonaducts and external genitalia in males depends chiefly upon substances secreted by the fetal testes These substances fall into two categories One causing regression of the Mullerian ducts and the other male development of the Wolffian ducts and masculinization of the external geni talia Known androgenic hormones can mimic the latter effect but do not cause regression of the Mullerian ducts

The essential defect in sexual development in this patient could be explained in terms of a failure of the embryonic testes to suppress differentiation of the Mullerian ducts Con ceivably such an event could have resulted from either the elaboration of a genetically in effective Mullerian duct suppressor substance or a genetic inability of the target organ to respond to normal suppressor substance These pathogenetic mechanisms have been evoked to explain a rare disorder of sexual development in which uterus and Fallopian tubes are found in apparently normal males These individuals have normal and well differentiated external male genitalia and the coexisting uterus Fal lopian tubes and vagina are usually an un expected finding at surgical repair of an in guinal hernia Hence the condition has been referred to as the uterine hernia syndrome or hernia uteri inguinale (1 3) The etiology of this condition is unknown but familial occur rence and parenteral consanguinity suggest the possibility of a genetic disorder (2)

Although hypospadias and scrotal abnormali ties are frequently associated with the D tri somy syndrome and with 4 p and 18 q-deletion syndromes we are not aware of cases in which ambiguity of the internal genitalia like that

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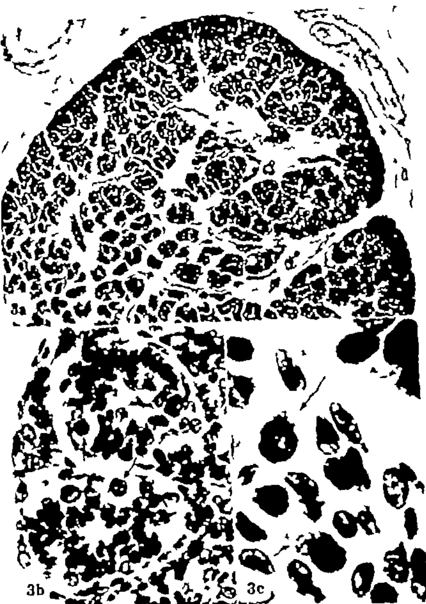


Fig. 3 (*a*) Testis showing well developed seminiferous tubules. H E × 35 (*b*) Detail showing spermatogonia close to the basement membrane (*arrows*) H E × 600 (*c*) Interstitial cells (Leydig's cells) in the inter tubular connective tissue (*arrows*) H E × 1 500

found to be ambiguous with a bicornuate uterus and rudimentary Fallopian tubes The testes and epididymis showed normal gross and microscopic appearance while the deferential ducts were hypoplastic and ended as Gartner's canals on both sides of the rudimentary utero-vaginal canal

The failure of the embryonic testes to suppress the Mullerian ducts in this patient is probably not a result of the genetic imbalance caused by the chromosome aberration but rather a fortuitous event related to the phenomenon encountered in the uterine hernia syndrome

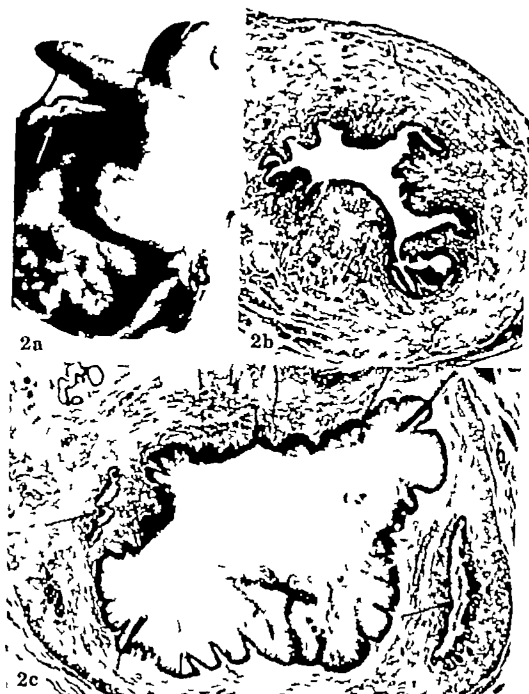


Fig 2 (*a*) Gross specimen of the persistent Mullerian organs The rudimentary Fallopian tubes are seen connected to the uterine cornua The arrow points to the left deferential duct just before it ends as a left Gartner's canal (Compare with Fig 1 and Fig 2 *c*) (*b*) Cross section of Fallopian tube H E ×35 (*c*) Uterovaginal canal cross section Note the highly stratified squamous epithelium The Gartner's canals at either side (*arrows*) are lined by a single layer of cylindrical cells H E ×35

present in our patient has been associated with abnormal autosomal chromosome complements This lack of similar cases in the literature favours the possibility that the failure of the embryonic testes to suppress Mullerian duct development in this patient was not caused by a general non specific effect of chromosomal imbalance, but rather a fortuitous event related to the phenomenon encountered in the uterine hernia syndrome

SUMMARY

A newborn patient with E trisomy syndrome and male pseudohermaphroditism is described At autopsy the internal genital organs were

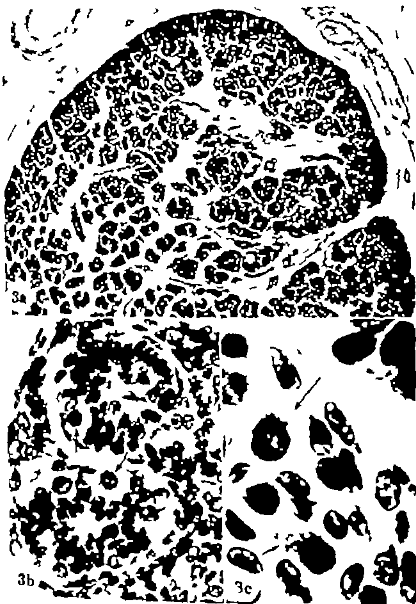


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PROCEEDINGS OF PAEDIATRIC SOCIETIES

NORWEGIAN PAEDIATRIC SOCIETY

Meeting Spring 1971

Ø Aagenæs *Intrahepatic/extrahepatic cholestasis differential diagnosis*

An early exact differential diagnosis between intra and extra hepatic cholestasis is important because

1 Extrahepatic cholestasis (biliary atresia) needs operation as soon as possible

2 Intrahepatic cholestasis should not be operated upon

Examinations that can help in the differential diagnosis are outlined The importance of examining the serum proteins and especially the $alpha_1$globulin to pick out the patients with $alpha_1$antitrypsin deficiency and Pi type ZZ is stressed

Familial occurrence is a strong indication of intrahepatic cholestasis as also is lymph oedema on the legs and a lymph screening might be appropriate

The radioactive Rose Bengal test is so far our best examination to differentiate between the intra and extra hepatic cholestasis An infant with a fecal excretion less than 10 % of the injected amount of Rose Bengal will probably have an atresia

The histology of the liver (needle biopsy) might give some help in differential diagnosis but seldom a very conclusive help

If this examination does not point to an intrahepatic cholestasis a surgical exploration must be performed

Meeting Dec 2 1971

Ø Aagenæs *Immunosuppressive therapy of liver diseases in childhood*

Immunosuppressive therapy mainly steroids has been used in different liver diseases neonatal cholestasis benign recurring cholestasis cirrhosis and liver insufficiency but in none of these conditions has this therapy proved to be of any benefit for the patients

On the other hand we have now good evidence that steroid therapy is of benefit for patients with chronic active hepatitis The works that support this thesis are summarized in the Copenhagen liver study and the recent study from Professor Sherlock's group at the Royal Free Hospital in London.

No conclusive evidence can be found for the benefit of treating patients with chronic active hepatitis with other immunosuppressive drugs but both Imurel and Purinethol have been used with a supposed success The treatment that we recommend for children with a chronic active hepatitis at the moment is prednisone 15 mg a day in bigger children 10 mg a day in smaller children if major pathology still persists mainly in serum albumin and gammaglobulin after some months of treatment Imurel is also added at a dosage of 1 mg kg per day If the treatment seems successful this treatment should probably be continued for 2–3 years

BOOK REVIEWS

J L. Melnick (ed) *Progress in medical virology* vol 13 S Karger Basel Munchen Paris London New York and Sydney 1971 503 pp US $26 40

The 1971 issue of Progress in Medical Virology is a worthy member of this distinguished series which nicely covers the current development within virology particularly from a clinical outlook The first two chapters of the present issue deal with more basic virological topics—new knowledge on replication of viral nucleic acids Bishop & Levintow review in their article the intermediate forms in RNA replication with emphasis on their structure and function They present results from studies on several virus systems and stress the need for investigation of viruses in mammalian cell systems Some problems may more easily be clarified by studies of the relatively less complex bacteriophages and small RNA viruses of animals Beyond them lie the questions of replication of the RNA containing tumor viruses and mechanism of oncogenesis—areas now receiving increasing attention

Phillips & Sydiskis review the results of investigations in vitro performed with cell free extracts Sub viral components are isolated and new knowledge is gained about the mechanism of viral nucleic acid replication The rapid development within this area is indicated by the fact that these two chapters require addenda to keep the reference up to date

Recent development within immunology has been of great importance also for virology and several chapters in this book deal with immune systems in relation to viral infections and viral vaccines P Ogra and D Karzon give an informative review on observations made in order to define the role of poliovirus antibodies in various body fluids in the pathogenesis of poliovirus infection The immunological response on natural infection and vaccination with live and inactivated vaccine is discussed The protective role of secretory antibody is considered for development of vaccines which would not only protect the vaccinated against disease but also provide an effective means of eradication of the virus in the human community

Rossen Kasel & Couch give an excellent summary of the evidence suggesting the presence of a secretory immunologic system They give a review of the antibody responses to active and inactivated respiratory viruses and discuss studies of vaccines applied locally in the respiratory tract Allergic reactions associated with viral vaccines are studied by Isacson & Stone In our days when vaccines are developed not only for life threatening diseases the authors point out that the health authorities must weigh carefully the risk of immunization against the risk of the disease itself

In a distinguished study of the antigenic variation of influenza virus Webster & Laver outline evidence suggesting that antigenic drift may result from changes in the amino acid sequence in the antigenic proteins They present experimental findings suggesting recombination between animal and human influenza viruses to be responsible for the abrupt appearance of the new strains producing pandemics

Porter assembles the information available about selected slow virus infections As yet these diseases are not adequately understood but they are now attracting increasing attention Human respiratory pathogens belonging to the corona virus group were first reported in 1965 These agents apparently cause a substantial proportion of human upper respiratory infections Bradburne & Tyrrell give an informative survey of the present knowledge of these agents

The first of the California group arboviruses was isolated back in 1943 from mosquitoes but not until 15 years later evidence of human diseases caused by such agents was recorded Clinical syndromes such as encephalitis aseptic meningitis and influenza like illnesses appeared both in the United States and Europe As diagnostic laboratories consequently began to include reagents for these viruses in their battery of tests it has become quite clear that California arboviruses represent a serious problem Henderson & Coleman review what is known today of these viruses In our country where RSSE is the only arbovirus so far recovered diseases borne by arthropods might deserve more attention

Since 1966 the Editor has included in each volume a chapter on virus classification giving an informative up to date outline of the scheme of animal virus classification Decisions made by the International Committee for Nomenclature of Viruses and its Subcommittees have been taken into account

Gun Carlstrom

C E Renfrew *Speech disorders in children* Pergamon Press Oxford 1972 69 pp £1 25

The book appears in the series Problems and Progress in Development edited by Dr J H Kahn The author is chief speech therapist at Churchill Hospital Oxford

According to the introduction "This small book is intended to help to understand the nature and scope of a speech therapist's work with children. It is not intended to instruct people on how to do it

In chapter one the scope of speech therapy is generally described and the three years of training in speech therapy in Great Britain briefly outlined. Chapter two deals with causes, diagnosis and treatment. The author gives a very good description of how something happens better than why. It is thus true that a deaf child has difficulties concerning the consonant sounds f th s sh but hardly because they are quiet. A much more likely reason is that these frequencies are located very high up in the sound spectrum while such children can best hear low tones. To take another example the difference of motivation one can find in a child if the parents have been talking to or with the child is very well put indeed. In the third chapter Management Advice and Treatment as in other parts of the book it is clear that the author is not only very experienced but also able to use her experiences with practical judgement. Chapter four Articulation Problems gives a general idea of how to deal with them while in chapter five is described some steps in the language development with skilful avoidance of all technical terms

Chapter six Disorders of Fluency This chapter is of necessity too short the problems of stammering (or stuttering) being too complex and too full of contradictions to be treated within a few pages. These considerations together with the fact that treatment of stammering is for the beginner very easy and for the more experienced speech therapist very difficult could be the reason why the author tries to avoid too definite opinions on the matter on the whole. At least when it comes to children less than ten years old however the author seems to prefer advice to the parents combined with the language teaching to the child i.e. the mother tongue (In this book as in so many other books within the field the words speech and language are used synonymously which is hardly quite correct to do as the difference is important. What is called articulation disorder should often be treated as language disorder)

Chapter seven Special Cases. Very little is said about voice and voice disorders somewhat more about aphasia and cleft palate. CP mental deficiency emotional disturbance and psychosis are briefly mentioned. From a certain point of view these cases may be described as special but as they are by no means few it would have been very well justified to devote them more attention

In conclusion the author stresses the importance of taking care not only of the technical side of speech but also of the support from the surroundings of the child

Speech disorders in children and speech therapy are surprisingly little known to surprisingly many of those who work with children which is a good reason to feel sorry for the children. This book is without reservation recommended to those who wish an introduction into the field of speech therapy

Sö en Fex

E. I. Issel *Bedeutung der zerebralen Geburtsschaden fur die Entwicklung des Kindes in der Gesellschaft* VEB Georg Thieme Leipzig 1971 215 pp DM 43.50

This book gives an analysis of various obstetric factors known to cause cerebral illness in the newborn. It correlates obstetric complications with mental retardation, cerebral palsy and behaviour disturbances. The study also tries to pay attention to other causes of cerebral disease i.e. genetic and social factors

The material is composed of 1 067 children born in the Women's Clinic of the Humboldt University in Berlin in the years 1950–61 which is about 10% of all the children born there during this period and is considered to be a representative sample with regard to the obstetric complications

The study is interesting because it gives us the late results in children closely observed in the perinatal period. It is however a rather old material and the obstetric complications were more serious at that time than nowadays. For instance Caesarian section was made in very few cases. There is also a remarkably large number of children classified as not normal

The results are compared with those of many other authors and the reference list is extensive. The problem is discussed mostly from an obstetrical point of view and the book is worth reading for those especially interested in the perinatal period

Ingrid Bjerre

S. Babson & R. C. Benson *Management of high risk pregnancy and intensive care of the neonate* 2nd ed C. V. Mosby Co. Saint Louis 1971 313 pp illus $16.50

In the United States perinatal mortality accounts for 100 000 deaths each year. More than 25% of these deaths would have been preventable if optimal care had been available. Complications of pregnancy and delivery will contribute to at least 100 000 mentally retarded and to another 200 000 with difficulties in school because of being "poor learners". These facts and the many problems related to perinatal medicine concerning diagnosis, perinatal care, therapeutic procedures as well as interdisciplinary communication are presented in this second edition of "Primer on prematurity and high risk pregnancy". The greatest improvement in comparison with the first edition is no doubt that the authors are dealing with the care of all newborn babies (not only premature infants) who are at risk and who should receive special intensive care in order not only to lessen perinatal mortality but also to increase the quality of survivors

The advantages and drawbacks of obstetric analgesia are dealt with in an instructive and commendable way. The appendix with definitions and terms is praiseworthy and terms like dysmaturity, postmaturity etc. are used in a consistent manner. In the chapter on respiratory insufficiency CPAP (continuous positive airway pressure) treatment is mentioned but no description of the technique is presented. Otherwise all therapeutic procedures of major importance in neonatal intensive care are instructively presented

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Recent development within immunology has been of great importance also for virology and several chapters in this book deal with immune systems in relation to viral infections and viral vaccines P Ogra and D Karzon give an informative review on observations made in order to define the role of poliovirus antibodies in various body fluids in the pathogenesis of poliovirus infection The immunological response on natural infection and vaccination with live and inactivated vaccine is discussed The protective role of secretory antibody is considered for development of vaccines which would not only protect the vaccinated against disease but also provide an effective means of eradication of the virus in the human community

Rossen Kasel & Couch give an excellent summary of the evidence suggesting the presence of a secretory immunologic system They give a review of the antibody responses to active and inactivated respiratory viruses and discuss studies of vaccines applied locally in the respiratory tract Allergic reactions associated with viral vaccines are studied by Isacson & Stone In our days when vaccines are developed not only for life threatening diseases the authors point out that the health authorities must weigh carefully the risk of immunization against the risk of the disease itself

In a distinguished study of the antigenic variation of influenza virus Webster & Laver outline evidence suggesting that antigenic drift may result from changes in the amino acid sequence in the antigenic protein They present experimental findings suggesting recombination between animal and human influenza viruses to be responsible for the abrupt appearance of the new strains producing pandemics

Porter assembles the information available about selected slow virus infections As yet these diseases are not adequately understood but they are now attracting increasing attention Human respiratory pathogens belonging to the corona virus group were first reported in 1965 These agents apparently cause a substantial proportion of human upper respiratory infections Bradburne & Tyrrell give an informative survey of the present knowledge of these agents

The first of the California group arboviruses was isolated back in 1943 from mosquitoes but not until 15 years later evidence of human diseases caused by such agents was recorded Clinical syndromes such as encephalitis aseptic meningitis and influenza like illnesses appeared both in the United States and Europe As diagnostic laboratories consequently began to include reagents for these viruses in their battery of tests it has become quite clear that California arboviruses represent a serious problem Henderson & Coleman review what is known today of these viruses In our country where RSSE is the only arbovirus so far recovered diseases borne by arthropods might deserve more attention

Since 1966 the Editor has included in each volume a chapter on virus classification giving an informative up to date outline of the scheme of animal virus classification Decisions made by the International Committee for Nomenclature of Viruses and its Subcommittees have been taken into account

Gun Carlstrom

C E Renfrew *Speech disorders in children* Pergamon Press Oxford 1972 69 pp £1 .5

The book appears in the series Problems and Progress in Development edited by Dr J H Kahn The author is chief speech therapist at Churchill Hospital Oxford

Acta Pædiat Scand 62 449–457 1973

PERCENTILES OF BIRTH WEIGHTS OF SINGLE LIVE BIRTHS AT DIFFERENT GESTATION PERIODS

Based on 125 485 births in Norway 1967 and 1968

TOR BJERKEDAL LEIV BAKKETEIG[2] and EGIL H LEHMANN[3]

From the Institute of Hygiene and Social Medicine University of Bergen Bergen Norway

In recent years newborns having an exceptionally low or high birth weight in relation to gestation period have been in the focus of interest These newborns are commonly referred to as small for date respectively large for date and are found to have a specially high risk of developing certain diseases (6 10 15–18) Identification is necessary from the point of view of primary prevention and early diagnosis This is dependent on a suitable reference material on birth weight in relation to gestation period

The commonly used reference materials in Norway are those published by Engström & Sterky (5) and Lubchenco et al (9) though others are also available (3 13 14) Engström & Sterky based their material on live births in Sweden during 1956–57 a total of 58 984 after exclusion of births with certain pathological conditions affecting mother or child and births for which length of gestation period was uncertain Lubchenco and co-workers based their material on 5 635 live births admitted to Colorado General Hospital during the years of 1948 to 1961 They did not include newborns with gross pathological conditions or newborns for which birth weight was not compatible with gestation period

The general use of these materials is limited however in view of the fact that human reproduction foetal growth and the condition of the newborn are influenced by social cultural and environmental characteristics that vary from one society to another and from one generation to another There is thus an obvious need to redefine standards of reference from time to time In Norway this has been made a relatively easy task through the foundation in 1970 of a National Medical Birth Registry The material of this registry permits an analysis of the relation between birth weight and gestation period in a total population of newborns Results of this analysis will be presented and the use of the material for reference will be discussed

MATERIAL AND METHODS

A national scheme of medical registration of births was introduced in Norway on January 1st 1967 (2, 11) The responsibility for central processing and analysis of the data collected through the scheme is placed by the Norwegian Health Services with the Medical Birth Registry which is a part of the Institute of Hygiene and Social Medicine University of Bergen Data on birth weight and gestation period for births registered during the years 1967 and 1968 a total of 135 731 have been utilized in the present

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Professor Head of the Institute of Hygiene and Social Medicine University of Bergen

Assistant professor Institute of Hygiene and Social Medicine University of Bergen

Research fellow (Norwegian Council for Science and the Humanities) EDP-Section of The Medical Faculty University of Bergen

This book ought to be available on the book shelf of every pediatric and obstetric clinic where newborn babies are cared for The facts and figures presented by Babson & Benson will be most useful in the training of doctors and nurses as well as for explaining and emphasizing to the responsible authorities the preventive aspect of perinatal medicine

Nils W Svenningsen

W Hamilton *Clinical paediatric endocrinology* Butterworths London 1972 209 pp £5 80

In 1950 the first edition of Lawson Wilkins The diagnosis and treatment of endocrine disorders in childhood and adolescence was published It is hardly an exaggeration to regard this event as the birth of paediatric endocrinology This remarkable textbook certainly served as a great stimulus for systematic clinical work and research in a field whose importance had not been previously fully realized Since then paediatric endocrinology has come into its own a fact which is also reflected by an increasing number of monographs and textbooks

The latest contribution is Clinical Paediatric Endocrinology by William Hamilton The author has for many years worked in this special branch of paediatrics but has as stated in the foreword remained a paediatrician His book is the second in a postgraduate paediatric series and written for the practising paediatrician The author has tried to use the same layout in each chapter—a short introduction describing the anatomy and physiology of the gland followed by an introduction of the function tests used today in clinical practice and finally a description of the disorders and syndromes associated with that particular gland This makes on the whole easy reading though the reader sometimes has difficulty in getting a comprehensive view of the clinical problem Precocious puberty is for instance referred to in at least five different parts of the book Some statements may be questioned e g that craniopharyngiomas frequently give rise to sexual precocity In the chapter on hypoglycaemia one misses a description of the idiopathic type with defective epinephrine response

The criticism presented here is however of minor importance and the book must be regarded as an excellent condensation of our present knowledge within the field of paediatric endocrinology Paediatricians not specialized in this branch of medicine will find it highly useful in practice

C G Bergstrand

E B Singleton & M L Wagner *Radiologic atlas of pulmonary abnormalities in children* W B Saunders Co Philadelphia London & Toronto 1971 251 pp illus £5 75

Lung disease in infancy and childhood frequently presents difficult diagnostic problems for the radiologist and has therefore been an important topic of instruction courses in pediatric radiology In the preparation of such courses given at meetings of the American Roentgen Ray Society Dr Singleton and Dr Wagner collected a vast material of illustrative cases which they have now utilized in a radiologic atlas of pulmonary abnormalities

The radiographs reproduced in this volume cover a variety of unusual diseases as well as common affections of the lungs with particular emphasis on congenital and acquired abnormalities in newborns and on pulmonary infections in infants and young children With a few exceptions the reproductions are of high quality and illustrate clearly the representative radiologic findings in the numerous conditions dealt with

Though essentially an atlas the book contains a considerable amount of reading with textual comments not only on the radiology but also on clinical aspects of the various abnormalities An appropriate number of references add to its value both to the general radiologist and to the specialist in pediatric radiology

G Theander

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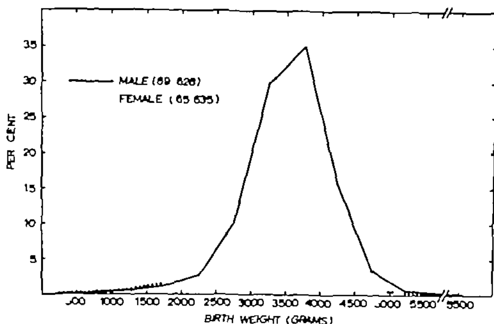


Fig 1 Frequency distributions of birth weights of male and female births in Norway 1967 and 1968

analysis with the exception of 36 births for which sex could not be determined

Of all births in Norway 98–99 take place in *an institution of which there are close to 200* This number includes small nursing homes as well as university clinics and maternity hospitals In these institutions birth weight is measured in grams on table scales According to custom the weights are rounded off upwards to the nearest number divisible by 10 The errors in this type of routine weighing do not appear to be too serious (4)

For births registered during 1967 and 1968 weights are known for *135 263* or *99 7* The distributions of birth weights for each sex separately are given in Fig 1 The distributions are unimodal having a slight *skewness towards lower birth weights Approximately* 95 of the birth weights were between 2 000 and 5 500 grams

Gestation *period is estimated from the date of* the first day of the last menstruation to the day of birth If beginning middle or end of a month was recorded on the registration form instead of the exact date of the beginning of last menstruation the date was set to the 5th the 15th or the 25th of the

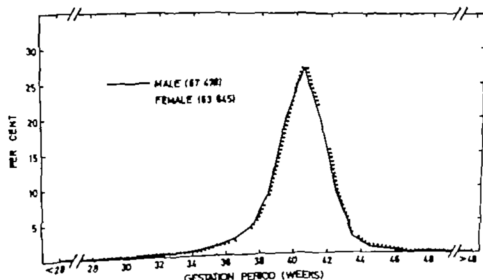


Fig 2 Frequency distributions of male and female births according to gestation period *Births in Norway* 1967 and 1968

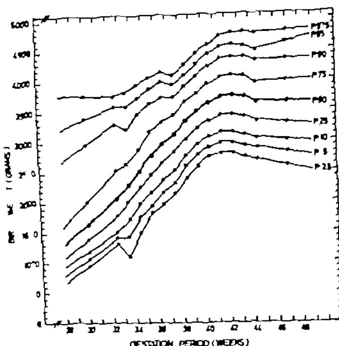


Fig 3 Percentiles of birth weights by gestation period for male single live births Based on 64 582 births in Norway 1967 and 1968

month respectively The timespan between the date of the beginning of the last menstruation and the date of birth is expressed in number of weeks to one decimal place Gestation period could be estimated for 131 123 or 96.6 of the total number of births The distributions of gestation periods for each sex separately are given in Fig The distributions can be seen to be unimodal with a maximum at 40–41 weeks of gestation and with a slight skewness towards shorter gestation periods Approximately 95% of the births took place after a gestation period of between 35 and 44 weeks

Of the 130 892 births in 1967 and 1968 with known sex and with known birth weight and gestation period only single live births with an estimated gestation period of 28.0 to 48.9 weeks have been included in the analysis of the relation between birth weight and gestation period A total of 125 485 births fulfilled these criteria of which 64 582 (51.5%) were males and 60 903 (48.5%) were females

The relation between birth weight and gestation period is presented in the form of percentiles of birth weights at different gestation periods Percentiles for each gestation period were found by computing the rank number M corresponding to a specified percentile by the formula $M = p(N+1)/100$ with p being the value of the percentile N the number of birth weights Birth weights were ranked in ascending order of weight and the weight having rank number M was taken as the p percentile (8) When M was not an integer linear interpolation between the next lower and the next higher weights was performed In the diagrams and tables the percentiles of birth weights given for each gestation period are 2.5 5 10 25 50 75 90 95 and 97.5

Birth Weight in Relation to Gestation Period for Male and Female Births

Percentile lines for birth weights of single live births with a gestation period of 28.0 to 48.9 weeks are presented in Fig 3 for males and in Fig 4 for females

The 50 percentile line (the line connecting median birth weights at various periods) indicates that the increase in birth weight from the 28th to the 40th week of gestation is almost linear both in male and female births The increase averages a little less than 200 g a week After 41st/42nd week of gestation there appears to be no further increase in median birth weight

As it will be seen from Figs 3 and 4 the percentile lines are in general parallel to and symmetrically located around the 50-percentile line for gestation periods of 37 weeks or more This indicates that birth weights for these gestation periods are distributed symmetrically around the median and that the variation in birth weights at each gestation period is of the same order of magnitude For male births interquartile range varies from 570 to 690 g for female births from 560 to 680 g

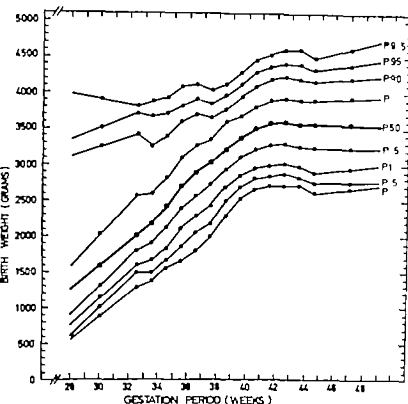


Fig 4 Percentiles of birth weights by gestation period for female single live births Based on 60 903 births in Norway 1967 and 1968

For pregnancies of shorter duration than 37 weeks the 90 95 and 97 5 percentile lines can be seen to diverge from the median line This indicates that the distributions of birth weights become more skewed towards larger weights the shorter the gestation period A similar trend has been reported by others (1 5 7) and may be caused at least partly by an underestimation of the gestation period It has been shown that the effect of employing stricter criteria for the estimation of gestation period is a diminishing and even a disappearance of the skewness of the weight distributions for the shorter gestation periods (7)

Effect of sex on relation between birth weight and gestation period

A comparison of the relation between birth weight and gestation period in the two sexes reveals that the percentile lines for male births run roughly parallel to and above the corresponding percentile lines for female births For the same gestation period birth weights of male births apparently average about 150 g more than that of female births

Effect of parity on relation between birth weight and gestation period

Of the several factors other than gestation period and sex that are known to influence birth weight (12) parity appears to be most significant The effect of parity on the relation between weight and gestation period is illustrated in Fig 5 Shown in Fig 5 are the 10 50 and 90 percentile lines for 24 585 first born and for 39 997 second and subsequently born male single live births The percentile lines for the total groups of male single live births are also included Judged by the 50-percentiles for gestation periods of 35 weeks and longer the effect of parity is to increase the birth weight by about 170 g for the second and subsequently born compared with the first born For female single live births the increase is about 130 g

Effect of pathology of pregnancy or birth on the relation between birth weight and gestation period

The material at hand although relatively large does not permit detailed analyses of the effect

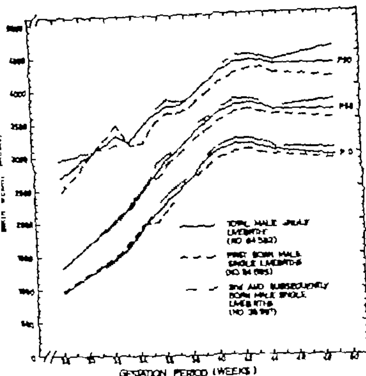


Fig 5 Percentiles of birth weights by gestation period and mother's parity for male single livebirths Births in Norway 1967 and 1968

on birth weight of specific pathological conditions of pregnancy and congenital conditions of the newborn. The gross effect of such conditions on the relation between birth weight and gestation period may however be investigated by the successive exclusion of births with congenital malformations, of births in mothers with either diabetes or toxemia and of births for which any pathology in mother or infant has been noted. The effect observed is illustrated in Fig. 6 by comparison of the 10, 50 and 90-percentile lines for the total group of 64 582 male single live births and the subgroup of 44 558 births without congenital malformation, disease or birth injury and without registered disease of the mother. The effect can be seen to be negligible down to a gestation period of 37 weeks. For shorter gestation periods some difference in birth weights becomes apparent, increasing as the gestation period becomes shorter. For a gestation period of 34 weeks the median birth weight of the subgroup of "normal" births is about 100 g higher than the median of the total group.

Use of the Material for Reference

The purpose of the analysis of the relation between birth weight and gestation period of single live births has been to define and describe a reference material suitable for identification of newborns that are "small for date" or "large for date". It has been found that the effect on the relation of excluding births with a history of pathology in the mother or the newborn itself is immaterial. No exclusions of single live births have therefore been made for reasons other than lack of information on sex, birth weight and gestation period and for extreme gestation periods. Thus the reference material may truly be said to be based on an unselected total population of single live births.

The analysis revealed that the effect on birth weight of sex and parity is in the main independent of gestation period. At the same gestation period the birth weight of male births averages about 150 g more than female births. Irrespective of gestation period the birth weight of the second and subse

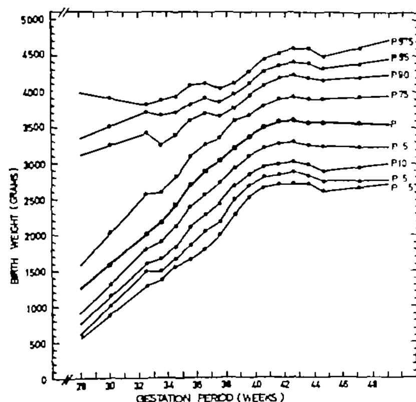


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The material at hand although relatively large does not permit detailed analyses of the effect

Table 1. Percentiles of birth weights by gestation period for male single livebirths

Based on 64 582 births in Norway 1967 and 1968.
Note. Before consulting the table 100 g should be added to the observed birth weight of a first born, 70 g should be subtracted from the birth weight of a second and subsequently born, provided gestation period has been estimated to be at least 35 weeks.

Gestation period (weeks)	No. of births	Percentiles (g) and 90% confidence intervals for the procent value (*P*) of the percentiles								
		p = 2.5	*p* = 5	*p* = 10	*p* = 25	*p* = 50	*p* = 75	*p* = 90	*p* = 95	*p* = 97.5
28.0–31.9	411	1.4–3.9 946	3.4–6.9 1 075	7.7–12.5 1 200	21.6–28.6 1 380	46.0–54.0 1 650	71.4–78.4 2 070	87.4–92.3 2 968	93.1–96.6 3 400	96.1–98.6 3 774
32.0–32.9	218	1.0–4.4 1 305	2.8–7.6 1 410	6.9–13.5 1 519	20.3–30.0 1 770	44.5–55.5 2 075	70.0–79.7 2 530	86.5–93.1 3 301	92.4–97.2 3 601	95.6–99.0 3 770
33.0–33.9	298	1.2–4.1 1 100	3.1–7.2 1 428	7.3–13.0 1 719	21.0–29.2 2 008	45.3–54.7 2 290	70.8–79.0 2 652	87.0–92.7 3 203	92.8–96.9 3 602	95.9–98.8 3 838
34.0–34.9	463	1.4–3.8 1 516	3.5–6.8 1 766	7.8–12.4 1 968	21.8–28.4 2 260	46.2–53.8 2 530	71.6–78.2 2 890	87.6–92.2 3 464	93.2–96.5 3 740	96.2–98.6 3 958
35.0–35.9	814	1.7–3.5 1 822	3.8–6.3 1 958	8.3–11.8 2 170	22.5–27.6 2 460	47.1–52.9 2 785	72.5–77.5 3 180	88.2–91.7 3 635	93.7–96.2 3 883	96.5–98.3 4 063
36.0–36.9	1 343	1.8–3.2 1 966	4.1–6.0 2 172	8.7–11.4 2 380	23.1–27.0 2 690	47.8–52.2 2 970	73.0–76.9 3 330	88.6–91.3 3 736	94.0–95.9 3 994	96.8–98.2 4 154
37.0–37.9	2 517	2.0–3.0 2 190	4.3–5.7 2 330	9.0–11.0 2 540	23.6–26.4 2 850	48.4–51.6 3 140	73.6–76.4 3 450	89.0–91.0 3 750	94.3–95.7 3 950	97.0–98.0 4 102
38.0–38.9	5 873	2.2–2.9 2 400	4.5–5.5 2 600	9.4–10.7 2 770	24.1–25.9 3 040	48.9–51.1 3 330	74.1–75.9 3 660	89.3–90.6 3 920	94.5–95.5 4 100	97.1–97.8 4 272
39.0–39.9	12 972	2.3–2.7 2 670	4.7–5.3 2 800	9.6–10.4 2 970	24.4–25.6 3 240	49.3–50.7 3 520	74.4–75.6 3 810	89.6–90.4 4 100	94.7–95.3 4 290	97.3–97.7 4 470
40.0–40.9	17 883	2.3–2.7 2 760	4.7–5.3 2 910	9.6–10.4 3 080	24.5–25.5 3 350	49.4–50.6 3 650	74.5–75.5 3 950	89.6–90.4 4 250	94.7–95.3 4 450	97.3–97.7 4 600
41.0–41.9	12 934	2.3–2.7 2 814	4.7–5.3 2 980	9.6–10.4 3 150	24.4–25.6 3 430	49.3–50.7 3 740	74.4–75.6 4 050	89.6–90.4 4 360	94.7–95.3 4 550	97.3–97.7 4 740
42.0–42.9	5 977	2.2–2.9 2 870	4.5–5.5 2 980	9.4–10.7 3 160	24.1–25.9 3 490	48.9–51.1 3 770	74.1–75.9 4 100	89.3–90.6 4 400	94.5–95.5 4 600	97.1–97.8 4 790
43.0–43.9	1 700	1.9–3.2 2 730	4.2–5.9 2 970	8.8–11.2 3 100	23.3–26.7 3 400	48.0–52.0 3 730	73.3–76.7 4 070	88.8–91.2 4 390	94.1–95.8 4 570	96.8–98.1 4 800
44.0–44.9	628	1.6–3.6 2 708	3.7–6.5 2 900	8.1–12.0 3 099	22.2–27.9 3 353	46.7–53.3 3 660	72.1–77.8 3 978	88.0–91.9 4 341	93.5–96.3 4 500	96.4–98.4 4 783
45.0–48.9	351	1.5–3.7 2 602	3.6–6.6 2 830	8.0–12.2 3 012	22.0–28.1 3 310	46.5–53.5 3 650	71.9–78.0 4 000	87.8–92.0 4 350	93.4–96.4 4 630	96.3–98.5 4 804

should be added before consulting Table 1 to find that this adjusted birth weight corresponds to the 5 percentile.

An observed or adjusted birth weight does not as a rule correspond to one of the percentiles given in Tables 1 or 2. Linear interpolation may be performed to find the percentile to which a weight corresponds. The error introduced by using linear interpolation rather than more elaborate procedures is supposed to be less than 0.5 between the percentiles 2.5 to 10 and 90 to 97.5 and less than 1.0 in the interval between the 10th and the 90th percentiles. These errors would seem quite acceptable in view of the sampling error attached to the tabulated percentiles.

Comparison of the Reference Material with Others

A comparison of the present material with that of Lubchenco et al. (9) shows that at corresponding gestation periods the birth weights of the present material are on the average

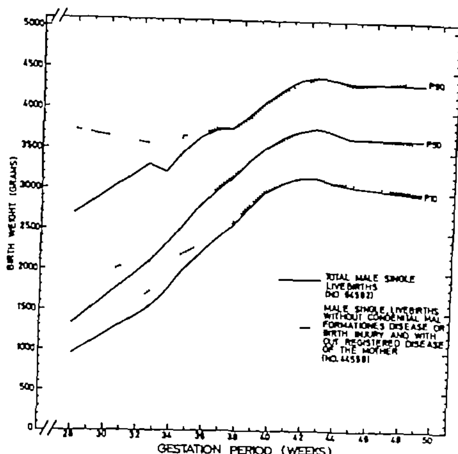


Fig 6 Percentiles of birth weights by gestation period for male single livebirths Total group and a selected group of births without malformation/disease/injury or registered disease of mother Births in Norway 1967 and 1968

quently born male single live birth averages about 170 g more than the first born the second and subsequently born female single live birth 130 g more than the first born These constant differences in birth weights make it possible to find the correct percentile of birth weight from one and the same reference table for all single live births by making the appropriate adjustment in birth weight for sex and parity For easy reference however the material is divided by sex and is presented in Table 1 for male births and in Table 2 for female births

In addition to the birth weights in grams that correspond to specified percentiles the tables include the 90 confidence limits for the percentiles The purpose of the 90 confidence limits is to give a measure of the precision of the percentile values E g a male birth of 32 weeks of gestation weighing 1 520 g may be seen from Table 1 to have a birth weight that corresponds to the 10 percentile The 90% confidence limits are 6 9 and 13 5 which indicate that the weight of 1 520 g for a male birth of 32 weeks of gestation would have corresponded in 90 of reference materials similar to the present to a percentile within the range of 6 9 to 13 5 In 10% of reference materials of the same size as the present and derived from the same population of births the birth weight would have corresponded to a percentile outside this range

The adjustment in birth weight to be made for a first born and a second and subsequently born in order to find from the tables the percentile to which the birth weight of a new born with known gestation period corresponds is quite simple If the gestation period is 35 weeks or longer 100 g should be added to the birth weight of a first born male 80 g to a first born female For a second and subsequently born male single live birth 70 g should be subtracted for a female 50 g If for example the birth weight of a first born male single live birth born after a gestation period of 40 weeks is found to be 2 810 g 100 g

about 200 g a week At the same gestation period male births were found to be on the average about 150 g heavier than female births Parity also had a notable influence on birth weight For gestation periods of 35 weeks and more the birth weight of a first born male was found to average about 170 g less than that of a second and subsequently born For female births the difference was 130 g

The material is suggested for use as reference for birth weight at various gestation periods and tables are included for this purpose

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Table 2 *Percentiles of birth weights by gestation period for female single livebirths*

Based on 60 903 births in Norway 1967 and 1968
Note Before consulting the table 80 g should be added to the observed birth weight of a first born 50 g should be subtracted from the birth weight of a second and subsequently born provided gestation period has been estimated to be at least 35 weeks

Gestation period (weeks)	No of births	Percentiles (g) and 90 confidence intervals for the percent value (*P*) of the percentiles								
		p = 2 5	*p* = 5	*p* = 10	*p* = 25	*p* = 50	*p* = 75	*p* = 90	*p* = 95	*p* = 97 5
28 0–31 9	273	1 2–4 2 879	3 0–7 3 1 000	7 2–13 1 1 134	20 8–29 4 1 300	45 0–55 0 1 590	70 6–79 2 2 020	86 9–97 8 3 242	92 7–97 0 3 506	95 8–98 8 3 904
32 0–32 9	177	0 9–4 7 1 283	2 6–7 9 1 498	6 6–13 9 1 598	19 8–30 5 1 790	43 8–56 2 2 010	69 5–80 2 2 575	86 1–93 4 3 408	92 1–97 4 3 706	95 3–99 1 3 806
33 0–33 9	229	1 1–4 4 1 365	2 9–7 6 1 485	7 0–13 4 1 670	20 4–29 8 1 905	44 5–55 5 2 180	70 2–79 6 2 595	86 6–93 0 3 260	92.4–97 1 3 660	95 6–98 9 3 873
34 0–34 9	353	1 3–4 0 1 556	3 3–7 0 1 677	7 5–12 7 1 850	21 3–28 9 2 130	45 6–54 4 2 400	71 1–78 7 2 815	87 3–92 5 3 388	93 0–96 7 3 713	96 0–98 7 3 933
35 0–35 9	678	1 6–3 6 1 660	3 7–6 4 1 860	8 2–12 0 2 109	22 3–27 8 2 390	46 8–53 2 2 695	72 2–77 6 3 100	88 0–91 8 3 590	93 6–96 3 3 811	96 4–98 4 4 086
36 0–36 9	1 049	1 8–3 3 1 800	3 9–6 2 2 055	8 5–11 6 2 280	22 8–27 2 2 560	47 5–52 5 2 900	72 8–77 2 3 260	88 4–91 5 3 700	93 8–96 1 3 900	96 7 98 2 4 115
37 0–37 9	2 017	2 0–3 1 2 000	4 2–5 8 2 185	8 9–11 1 2 430	23 4–26 6 2 730	48 2–51 8 3 030	73 4–76 6 3 350	88 9–91 1 3 650	94 2–95 8 3 840	96 9–98 0 4 030
38 0–38 9	4 841	2 1–2 9 2 301	4 5–5 5 2 490	9 3–10 7 2 680	24 0–26 0 2 940	48 8–51 2 3 220	74 0–76 0 3 500	89 3–90 7 3 770	94 5–95 5 3 960	97 1–97 9 4 120
39 0–39 9	11 746	2 3–2 7 2 540	4 7–5 3 2 690	9 5–10 5 2 850	24 3–25 7 3 100	49 2–50 8 3 380	74 3–75 7 3 670	89 5–90 5 3 950	94 7–95 3 4 120	97 3–97 7 4 280
40 0–40 9	17 593	2 3–2 7 2 660	4 7–5 3 2 810	9 6–10 4 2 960	24 5–25 5 3 210	49 4–50 6 3 500	74 5–75 5 3 800	89 6–90 4 4 090	94 7–95 3 4 290	97 3–97 7 4 450
41 0–41 9	13 072	2 3–2 7 2 700	4 7–5 3 2 840	9 6–10 4 3 000	24 4–25 6 3 280	49 3–50 7 3 580	74 4–75 6 3 890	89 6–90 4 4 190	94 7–95 3 4 360	97 3–97 7 4 530
42 0–42 9	5 948	2 2–2 9 2 710	4 5–5 5 2 880	9 4–10 7 3 030	24 1–25 9 3 300	48 9–51 1 3 600	74 1–75 9 3 920	89 3–90 6 4 230	94 5–95 5 4 410	97 1 97 8 4 590
43 0–43 9	1 751	1 9–3 1 2 708	4 2–5 9 2 830	8 8–11 2 2 980	23 3–26 7 3 260	48 0–52 0 3 565	73 3–76 7 3 890	88 8–91 2 4 190	94 1–95 8 4 390	96 9–98 1 4 592
44 0–44 9	637	1 6–3 6 2 600	3 7–6 5 2 750	8 1–12 0 2 888	22 2–27 9 3 240	46 7–53 3 3 570	72 1–77 8 3 900	88 0–91 9 4 160	93 5–96 3 4 310	96 4–98 4 4 481
45 0–48 9	539	1 5–3 7 2 663	3 6–6 6 2 760	8 0–12 2 2 950	22 0–28 1 3 240	46 5–53 5 3 555	71 9–78 0 3 920	87 8–92 0 4 200	93 4–96 4 4 390	96 3–98 5 4 610

approximately 300 g higher As a result the 50 percentiles of the present material correspond roughly to the 75 percentiles in the material of Lubchenco A much closer correspondence is found when comparing the present material to that of Engstrom & Sterky (5) This was perhaps to be expected because the living conditions of the Swedish population in 1956 and 1957 when Engstrom & Sterky collected their material were probably not very different from those of the Norwegian population in 1967 and 1968

SUMMARY

A total number of 125 485 single live births with known sex and birth weight and a gestation period estimated to be from 28 0 to 48 9 weeks were registered in Norway in 1967 and 1968 Information about these births provided by a national scheme of medical registration of births is used in a study of the relation between birth weight and gestation period

An almost linear relation was found to exist for gestation periods of 35 to 42 weeks In this interval median birth weight increased

about 200 g a week At the same gestation period male births were found to be on the average about 150 g heavier than female births Parity also had a notable influence on birth weight For gestation periods of 35 weeks and more the birth weight of a first born male was found to average about 170 g less than that of a second and subsequently born For female births the difference was 130 g

The material is suggested for use as reference for birth weight at various gestation periods and tables are included for this purpose

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Acta Pædiat Scand 62 458–464 1973

RETROLENTAL FIBROPLASIA IN SWEDEN

General Survey and Selected Study on Patients born in 1960–1966

BJÖRN SVEDBERGH and EVA LINDSTEDT

From the Department of Ophthalmology (Head G von Bahr) Akademiska sjukhuset Uppsala Sweden

Around 1952 it was concluded that retrolental fibroplasia (RLF) was a mainly iatrogenic disease caused by excessive oxygen administration (38 48) Rigorous restrictions were introduced with a consequent dramatic fall of the incidence of RLF Later on however it was reported that the decrease of RLF was paralleled by an increase of mortality in hyalin membrane disease or respiratory distress syndrome (RDS) (7) and of incidence in spastic diplegia (16) leading to a more liberal view on oxygen administration

The present study was prompted on the one hand by the articles of Seedorff in 1968 (51 52) surprisingly showing the same total incidence of RLF in Denmark before and after 1952, and on the other hand by a definite impression of an increasing incidence of RLF in Sweden during the early 1960s, judging from the inventory of blindness performed by Lindstedt (39 40)

In Sweden the first case of RLF was reported by Hedlund Kristiansen in 1948 (21) However in 1947 Wadensten described a case of bilateral non attachment of the retina, which was probably RLF (58 59) Bjelkhagen presented 38 cases in 1952 (11) and Hellstrom 42 cases 1954–1956 (25) In 1963 one third of the pupils of Tomteboda Institute for the Blind had RLF (44) The appearance of the premature fundus of the eye at early RLF and oxygen administration was described by Huggert 1952–1954 (27 28 29) First in the world to report of experimental studies correlating RLF and oxygen were Gyllensten & Hellstrom in 1952 (20) and they further contributed to elucidate the pathogenesis (22, 23 24 and see 37) Review articles have been presented by Karlberg in 1955 (33) Karpe et al in 1957 (34) and concerning oxygen administration by Finnstrom in 1969 (17)

SCOPE OF THE SURVEY

Methods

An *inventory* has been made of all Swedish children born in the period 1945–1966 who suffered from RLF Data were collected from various sources Bjelkhagen s study (11) Lindstedt s (39 40) the register of the Swedish Board of Health (comprising most cases registered since 1952) and the files of the state schools for blind children

A *selected study* has been performed of those children born between 1960 and 1966 collecting information from the patient reports of th obstetric pediatric and ophthalmological clinics Special interest was focused on factors concerning oxygen administration

RESULTS

The inventory

The inventory comprises 274 patients with assumed RLF (Fig 1) An unknown number of patients with a reversible affection or with unestablished diagnosis are included The in

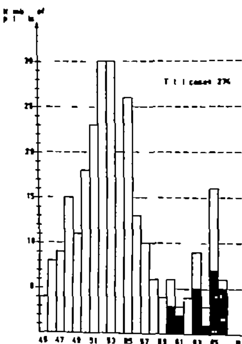


Fig 1 Cases of RLF in Sweden born in 1945–1966 Hatched columns represent 23 cases with irreversible RLF 1960–1966 dealt with in the present selected study

cidence shows a peak at about 1952–1953 a dramatic fall till 1960 and thereafter a gradual increase The latter trend can be partly explained by the 15 increase of prematurely born infants (i e birth weight less than 2 500 g) and by the 25 decrease in the 6 month mortality figures in all children from 1960 to 1966 (see 19 and 54)

The selected study

The selected study includes 46 children with assumed RLF (Fig 1) The disease showed a reversible course in 12 of the children (11 being reported from the same country town) and for another 11 children the diagnosis of RLF was not established (see below) The remaining 23 children with an established diagnosis of irreversible RLF were subjected to further study

Type of clinic In Bjelkhagen s study 1945–1950 (11) 20 patients were nursed at university clinics and 18 at other clinics whereas in the present study the number of patients were 2 and 21 respectively Whether this change can be fully explained by the spread of incubators and/or differences in perinatal care has not been possible to establish

Birth weight and gestational age 3 children had birth weights below 1 000 g 17 below 1 500 g and none had a birth weight above 2 250 g

From a patho-physiological point of view the gestational age of the child at birth is of greater interest Of 23 children 20 had a gestational age of 27–32 weeks (Fig 2) One child was born at full term and the history is interesting

Patient 1 B N boy 1965 Mother healthy primi grav pregnancy and delivery normal Full term birth birth weight 2 120 g sign of postmaturity and placenta partly calcified Oxygen supply only a few minutes post partum Hernia inguinale At 8 months of age first eye examination showed microphthalmos and RLF-changes in both eyes verified by pathological anatomical diagnosis of the right eye

Time of diagnosis The diagnosis had been established in all children within the first year of life but never within the first month In 7 patients no signs of RLF were observed on ophthalmic examination which had taken place during the first 3 months No one was diagnosed during oxygen administration

Oxygen administration All except patient 1 had been subjected to oxygen administration for 1–11 weeks (Fig 3) The modes of ad

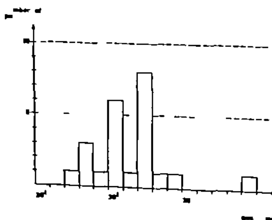


Fig 2 Gestational week at time of birth

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Patho-physiology The vascularization of the retina takes place up to the 32nd gestational week and this period is also the most susceptible period for developing RLF (8 48) in good agreement with our findings Vascularization may however continue up to full term and possibly explain cases of RLF at infants born at full term and receiving oxygen (14 46)

When oxygen was incriminated around 1952 it was at first believed that the sudden relative anoxia following oxygen administration was the primary causative factor in RLF (10 30 56 57 and see 48)

However later on it was shown histologically that the primary lesion in RLF was caused by the direct effect of oxygen administration Thus Ashton demonstrated a lesion to the growing vessel endothelium leading to vaso-obliteration both in animal (2 3 4) and man (5 18) Furthermore RLF was observed developing during oxygen administration (see 48)

Recently Lemmingson has beautifully demonstrated by vital microscopy that the initial vaso constriction seen at oxygen administration is in fact a rheological question The axial erythrocyte stream is diminished in calibre and the peripheral plasma is correspondingly increased the vessel calibre being unchanged This probably leads to hypoxia in the otherwise hyperoxygenated animal He also showed that gradual withdrawal of oxygen or re oxygenation had no therapeutic effect on RLF-changes rather the contrary (37)

In view of the present state of knowledge it must thus seem a highly debatable treatment for RLF to put an infant with clinically observable RLF-changes back into the incubator with oxygen administration since this could cause further damage to the growing vessel endothelium This reasoning also applies to the question of gradual withdrawal (37 48) Quite another aspect in this context are the toxic lesions of oxygen on the lung tissue (6 31 32 43) which necessitate a gradual withdrawal because of pulmonary malfunction

Intra uterine hypoxia followed at birth by the relative excess of oxygen in normal atmosphere has been suggested as a cause of RLF (12 25 26 53) The history of patient 1 in the present study might be added in support

Administration of oxygen The *indication* for oxygen administration is hypoxia as indicated by clinical condition or laboratory data Apnoic attacks or irregular breathing alone is no indication (8 48)

The *mode* of oxygen administration demands comment In about half the RLF-patients in both Seedorff s (51) and our study the oxygen had been administered part of the time by funnel The funnel usually emitting 100 oxygen was placed at varying distances from the face of the child with no concentration being measured in the breathing atmosphere sometimes for many days This cannot be considered a well controlled procedure This mode of administration should be reserved only for acute situations and then for the shortest time possible Of considerable interest are the experiments on kittens by Ashton & Garner (5 18) showing that intermittent oxygen administration can prevent permanent injury to the growing retinal vessels presumably by avoiding a cumulative toxic effect Similar findings are expressed in a clinical study by Kittel (35)

The duration of oxygen administration is a factor of considerable importance (36 37 and others) and it is noteworthy in our study that all children except patient 1 had oxygen for more than 1 week

Control of oxygen administration The control of oxygen administration is primarily based on *clinical condition* It is well to recall the words of Ballantyne & Michaelson (8) "Premature infants should not be placed in oxygen unless it is absolutely essential and then for the minimum length of time or of Patz (45) " in the minimum quantity for the shortest period consistent with the infant s survival

The concentration of oxygen in breathing atmosphere in the incubator is measurable and is or has been the most widely used though

ministration are classified according to the proposal of Seedorff (51) whose results are shown as a comparison in the table The oxygen was administered either by insufflation into the incubator (with some control of the concentration of oxygen in breathing air) or by a funnel placed in front of the child s face, in which case the oxygen concentration in breathing air was not under control In some children the first method alone was used and *it is noteworthy that for only* one *patient* did the oxygen concentration in breathing air exceed 40° *Often the child was subjected to* both methods but none was subjected to funnel alone *In this connection it may be added* that 3 children later received oxygen as treatment for RLF and that only one child had her arterial oxygen tension measured

Other factors investigated Other factors such as type of incubator transfusion of blood anemia, hyperbilirubinemia, mother s general health complications during pregnancy and delivery have been investigated Due to the small number of patients and lack of relevant comparison data any statistical analysis has been considered of doubtful value

The absolute frequencies of diseases other than RLF however may be of some interest Thus, only 4 patients suffered from respiratory distress syndrome *(RDS) 4 patients* had *hydro*cephalus and mental retardation was established *in 7 patients* or about *one third* (it should be noted that 18 patients were at the pre school age when this study was under taken in 1970) Cutaneous haemangioma was observed in 6 patients a figure higher than normally expected but in good correlation with earlier findings in RLF (53)

Concerning the indications for oxygen administration, the information in the patient reports has often been too scanty to permit a reliable analysis

Remarks on children with unestablished diagnosis of RLF The diagnosis of RLF was unestablished in 11 children For 2 patients patient reports were not obtainable, and in 6 patients diagnosis other than RLF were established Finally there were 3 children with congenital encephalo ophthalmic dysplasia which will be described in a separate paper (55)

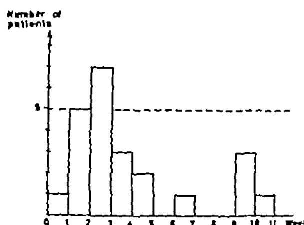


Fig 3 Duration of oxygen administration

DISCUSSION

The aim of this study has been to pinpoint some facts about the more recent cases of RLF in Sweden in the hope that some recommendations for better care of the newborn will result To satisfactorily explain the rising incidence *during the early sixties has not* been possible due to the influence of unregistered factors such as the spread of incubators number of oxygen treatments differing opinions on oxygen administration and so forth The selected study of the 23 irreversible RLF patients will be discussed with the emphasis on the role of oxygen

Table 1 *Modes of oxygen administration according to the classification by Seedorff (51)*

Mode of oxygen administration	Number of patients: Present study	Number of patients: Seedorff (-68)
No oxygen	—	4
Oxygen only at revival postpartum	1	—
Incubator O_2-conc <40	8	3
Incubator O_2-conc >40	1	3
Incubator O_2-conc not stated	2	4
Incubator and funnel	11	10
Funnel only	—	8
Mode of administration not stated	—	5
Total patients	23	37

After a dramatic decrease in incidence since 1953 there has been a slight increase between 1960 and 1966. Thus 23 patients with irreversible retrolental fibroplasia during this latter period as a rule were of very low birth weight and gestational age received oxygen for more than 1 week and in maximum concentrations below 40 in breathing air in the incubator. Oxygen was partly administered by funnel to half the patients. One patient history indicated a pathogenetic connection with intrauterine hypoxia. Aspects of pathophysiology oxygen therapy and its control are discussed.

ACKNOWLEDGEMENT

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somewhat overrated aid in control Animal experiments (2 23 37 46) as well as clinical reports around 1956 that a maximum concentration below 40% resulted in a large reduction in the incidence of RLF lead to a general belief that 40% was the "safe level although a contradictory view was stressed by the original authors (36) and others (15) In the present study only one patient received a maximum measured concentration exceeding 40° Moreover the ensuing strict limitation to 40 resulted in an increase in mortality of RDS (7) since the oxygen concentration in breathing air is often poorly correlated to the oxygen tension in the patient's blood and very high concentrations are often needed to manage these patients (3 48 50)

Arterial oxygen tension P_{aO} is the only adequate measurement but with the present technique demands great personnel and laboratory resources generally only available at advanced premature nurseries Furthermore umbilical arterial catheterization has its complications in 2–7% (50) and samples taken postductally are sometimes badly correlated to oxygen tension pre ductally due to shunting (6) Samples from the radial or temporal artery would be ideal but are technically difficult to obtain for any length of time

As yet one does not know for certain at which P_{aO} level RLF develops but great efforts are being made to solve this problem (1 9 13 41 42) and retinal vaso constriction has already been observed at a P_{aO} level of 100 mmHg (1) It is noteworthy that in the present study only one patient had her P_{aO} measured

Acid base examination might also be of help since an increasing metabolic acidosis with adequate ventilation (normal P_{CO}) indicates hypoxia The samples are taken from so called arterialized capillary blood (17)

Ophthalmologic examination is usually beset by difficulties in the very premature infant because of vitreous haze, persistent hyaloid artery and examination within or outside the incubator (38, 42) Usually the first stage of RLF according to the classification of Reese et al (49) namely dilatation and tortuosity of the vessels with localized areas of neovascularization and retinal oedema, are not observed until 5–10 weeks after cessation of oxygen administration (38) at a time when irreversible damage to the growing vessels may have occurred many days earlier Constriction and attenuation of the retinal vessels are considered to be a pre RLF stage (28 29 45 47 and others see however 37) In this situation Patz recommends immediate withdrawal of oxygen administration and if there is regression in about 15 minutes the prognosis should be good (45 47) Retinal vasoconstriction is considered a severe clinical sign and an ophthalmic control of oxygen administration should only be undertaken when there is no facility for P_{aO} measurement (1 42 47) However a normal appearance of the retinal vessels tells nothing about the actual arterial oxygen tension as demonstrated in recent studies (1 9 13) The Committee on Fetus and Newborn in the USA has recently given detailed recommendations for oxygen therapy in the newborn infant (15) It recommends regarding ophthalmic examination that a person experienced in recognizing retrolental fibroplasia (retinopathy of prematurity) should examine the eyes of all infants born at less than 36 weeks gestation or weighing less than 2 000 g (4 2 lb) who have received oxygen therapy This examination should be made at discharge from the nursery and at 3 to 6 months of age Thus it still seems an open question whether the ophthalmologist may be of any greater practical help in controlling oxygen administration

In the future refined ophthalmic examinations and a less demanding procedure for P_{aO} measurements may be important tools in navigating these infants between brain and lung damage blindness and death

SUMMARY

In Sweden 274 cases of retrolental fibroplasia have been reported between 1945 and 1966

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METABOLIC OBSERVATIONS IN INFANTS OF STRICTLY CONTROLLED DIABETIC MOTHERS

Plasma Levels of Glucose FFA Glycerol and D β hydroxybutyrate during the First Two Hours after Birth

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Lower than normal free fatty acids (FFA) concentrations have been reported in infants of diabetic mothers (IDM) and infants of gestational diabetic mothers (IGDM) during the first hours after birth (5 10 11 12 25 33 34) These observations have been interpreted to reflect indirectly a state of functional hyperinsulinism Simultaneous determinations of plasma FFA and glycerol have not been reported in IDM Since inhibition of lipolysis is regarded as one of the most insulin sensitive processes (2 8) and our previous studies of normal newborns (28 32) indicated that lipolysis increased immediately after birth as indirectly reflected in a rapid rise in glycerol concentration we decided to study this aspect of lipid metabolism in a large group of IDM and IGDM during the first hours after birth

In view of the concept that maternal hyperglycemia during pregnancy leads to fetal and subsequently postnatal hyperinsulinism (23) an attempt was made to relate the degree of metabolic control during pregnancy to the metabolic findings of the infants Although the prenatal care of the diabetic women was standardized it must be emphasized that the infants studied represented a heterogeneous group with respect to factors such as maternal age weight parity degree of diabetic angiopathy achieved degree of metabolic control acute complications during pregnancy maturity and birth weight of the infants

MATERIAL AND METHODS

Fifty seven infants were studied 37 infants of insulin dependent mothers (IDM) 15 infants of gestational diabetic mothers (IGDM) and 7 infants of non-diabetic mothers (controls) In all instances the parents were informed of the nature and purpose of the investigation before delivery and consent was obtained to study the infants Results from a group of 22 normal infants previously studied during the first 2 hours with comparable protocol were also used as control data (32)

Management of diabetic mothers

The insulin treated mothers of the infants studied formed a sub group of 1 0 mothers followed and treated in the same hospital in a similar manner during pregnancy According to WHO definitions the present perinatal mortality was 4 1

The pregnancies were classified according to White (37) Overweight before actual pregnancy was defined as more than 10% above the ideal weight for height using Scandinavian weight standard (4) Complications during pregnancy were classified according to Pedersen & Pedersen (4) The main principles of the treatment were as follows

1 To avoid premature interruption of pregnancy Pregnancy was allowed to continue to term but when evidence presented itself that fetal well being would be jeopardized pregnancy was terminated
2 To achieve normoglycemia i e fasting and post prandial blood glucose concentrations below 90 and 150 mg/100 ml respectively
3 To treat intensively acute complications such as pre eclampsia significant bacteriuria and pyelonephritis

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ablc 2 *Mean plasma values ± S D of FFA glycerol glucose and D-β hydroxybutyrate in IDMs GDMs and controls*

Infant group	Maternal vein	p[a]	Umbilical vein	p	Minutes after birth 30	p	60	p	90	120	p
FFA (mM)											
IDM	0 67 (29)[b] ±0 30	ns	0.23 (36) ±0 09	ns	0 19 (24) ±0 07		0 26 (33) ±0 21		0 30 (31) ±0 23	0 34 (30) ±0 23	
IGDM	1 19 (14) ±0 35		0 32 (15) ±0 07	ns	0 30 (10) ±0 16	ns	0 48 (13) ±0 26	ns	0 61 (10) ±0 34	0 63 (11) ±0 33	ns
Control	0 80 (7) ±0 13		0 28 (25) ±0 10		0 32 (27) ±0 21		0 61 (29) ±0 49		0 84 (3) ±0 17	0 80 (28) ±0 42	
Glycerol (mM)											
IDM	0 150 (31) ±0 067	ns	0 122 (37) ±0 074		0 181 (23) ±0 077	ns	0 229 (32) ±0 098	ns	0 254 (29) ±0 121	0 256 (28) ±0 120	ns
IGDM	0 81 (14) ±0 127		0 114 (15) ±0 040		0 206 (11) ±0 098	ns	0 284 (13) ±0 134	ns	0 299 (10) ±0 143	0 274 (12) ±0 125	ns
Control	0 154 (7) ±0 063		0 080 (24) ±0 079		0 176 (27) ±0 073		0 249 (29) ±0 118		0 228 (3) ±0 038	0 274 (28) ±0 095	
Glucose (mg/100 ml)											
IDM	141 8 (27) ±54 4	ns	101 6 (30) ±41 6		29 4 (20) ±16 7		1 9 (29) ±15 5		26 4 (28) ±17 8	29 0 (27) ±16 0	
IGDM	135 1 (12) ±51 6	ns	93 0 (13) ±22 8		46 2 (9) ±16 3	ns	44 0 (11) ±23 9	ns	48 8 (9) ±29 4	53 4 (11) ±28 0	ns
Control	108 4 (7) ±42 3		73 6 (24) ±24 0		57 4 (25) ±21 5		51 0 (29) ±18 3		52 3 (3) ±2 6	59 8 (27) ±12 9	
D β hydroxybutyrate (mM)											
IDM	0 499 (31) ±0 525	ns	0 259 (37) ±0 261	ns	0 056 (24) ±0 062	ns	0 042 (32) ±0 036		0 045 (30) ±0 050	0 049 (30) ±0 055	
IGDM	1 471 (13) ±0 890		0 795 (15) ±0 534		0 177 (9) ±0 177		0 074 (12) ±0 048	ns	0 081 (10) ±0 047	0 074 (11) ±0 059	ns
Control	0 308 (7) ±0 140		0 267 (24) ±0 176		0 066 (27) ±0 036		0 067 (29) ±0 059		0 080 (3) ±0 007	0 084 (29) ±0 065	

[a] p indicates significant differences between mean control values and corresponding mean values of IDM and IGDM ns – non significant
$p < 0.05$ $p < 0.01$ $p < 0.001$
[b] Figures in parentheses indicate the number of infants studied

50% glucose solution) was performed after an over night fast A diet containing at least 300 g of carbo hydrates had been prescribed for 3 days prior to the test The glucose disappearance rate was calculated on the absolute blood glucose concentrations determined on capillary blood samples by a glucose oxidase meth od (Glox AB KABI Stockholm Sweden) The glu cose disappearance rate was expressed as $100 \times k = k$ Mothers with a k below 1 13 (35) during pregnancy and in some cases confirmed at repeat tests and who had normal k values after delivery were diagnosed as gestational diabetics These mothers were treated with diet alone and were seen at regular outpatient visits and were usually hospitalized during the last weeks of pregnancy The gestational diabetic mothers were otherwise supervised in a similar manner d scribed above for insulin treated mothers Four of the mothers were delivered by cesarean section The ind cations were p rsistent fetal bradycardia in 2 and pre-eclampsia in (See also Table 1)

Mothers of control infants

The 7 mothers of the control infants had none of the above criteria used for selection of gestational diabetics and had normal k values during the last trimester of pregnancy Three of the mothers were delivered by cesarean section because of contracted pelvis

Study protocol

All infants studied had Apgar scores of 7 or more at 1 and 4 minutes after birth The infant was immedi ately placed below a radiant heat source and the up p r airways were carefully cleared of mucus by suction The infant was thereafter placed in an in cubator at an environmental temperature of 3 34 C Between 10 and 20 minutes after birth umbilical cathe ters were inserted under sterile conditions In most cases arterial catheters (5 French polyvinyl chloride catheter) were used In some cases both arterial and

Table 1 *Clinical data on mothers and infants*
Values are given as mean ± S D

	IDM (White classes)					
	B	C	D	Total (B+C+D)	IGDM	Control
Mother						
Number of cases	7	17	13	37	15	7
Age (years)	30 6±5 8	24 1±4 3	24 5±2 9	25 4±4 8	27 6±6 0	25 7±4 0
Height (cm)	165 9±6 1	163 2±5 4	166 9±5 6	164 9±5 7	166 4±4 8	163 1±8 8
Weight before actual pregnancy (kg)	67 1±23 6	56 8±4 1	59 5±7 6	59 6±11 5	64 9±13 9	55 4±10 2
Weight gain (kg)	6 6±7 9	11 0±3 5	11 2±3 7	10 5±4 6	9 8±7 6	13 7±3 1
Overweight before actual pregnancy (number)	2	0	0	2	3	0
Preeclampsia (number)	1	0	4	5	1	0
Mode of delivery						
Vaginally	3	2	3	8	11	4
Cesarean section	4	15	10	29	4	3
Infant						
Gestational age (days)[a]	273±6	263±12	263±7	265±10	277±8	285±14
Birth weight (kg)	3 66±0 37	3 36±0 68	3 47±0 54	3 45±0 59	3 89±0 45	3 51±0 38
Birth length (cm)	51 6±2 1	49 9±2 2	50 8±2 1	50 5±2 2	52 3±2 2	51 5±1 9
Birth weight percentile[b]	68±31	60±38	59±30	61±33	77±29	44±27
Sex (number) ♀	4	8	4	16	7	5
♂	3	9	9	21	8	2

[a] Gestational age was calculated from the date of the first day of the last menstruation
[b] Birth weight percentiles were calculated using Swedish standards (40)

Outpatient The mothers attended a special mothers welfare clinic and the first visit was usually between the 10th and 12th weeks of gestation Thereafter they were seen every 1 or 2 weeks during the first 2 trimesters and weekly during the 3rd trimester At each outpatient visit the urine from the preceding day and night was analysed for glucose protein and ketone bodies Weight and blood pressure haemoglobin and blood glucose at 8 and 14 hours were also measured Patients were also instructed about monitoring their diabetes with Clinitest® and Ketostix® (Ames Company England) The pregnant women and expectant fathers were given thorough instructions concerning the specific problems of diabetes and pregnancy The mothers were prescribed an individual diet with a daily intake of 1 200–2 000 kcal (range for series) The average amount of calories from carbohydrate fat and protein was 45 27 and 28 respectively

Hospitalization All women were hospitalized at least 3 times The first time was when pregnancy was diagnosed and then again about the 22nd week of gestation The women were routinely admitted to the hospital for about 1 week From the 32nd week all mothers stayed in hospital until delivery During hospitalization the patients were given a diet based on prefabricated deep frozen meals of known composition (Findus Bjuv Sweden) With the exception of a few cases most women were given 1 600 kcal per day Breakfast was served at 8 lunch at 12 snack at 3 dinner at 5 and snack at 8 o clock During each period in hospital the mothers were examined for signs of retinopathy nephropathy and bacteriuria Blood glucose was determined 5 times daily and urinary glucose was monitored on 12 hourly specimen Urinary oestriol determinations were performed at regular intervals (30) and during the *final period in hospital* fetal heart rate was recorded at least 4 times daily

Twenty nine women were delivered by cesarean section The indications were failure of induction in 5 persistent fetal bradycardia in 6 consecutive decreases in urinary oestriol excretion in 5 pre-eclampsia in 4 contracted pelvis in 5 elective for other reasons in 3 and miscellaneous in 3 The insulin dose was reduced approximately 50 12–24 hours prior to delivery and most mothers received a 10 glucose solution intravenously in order to keep the maternal blood glucose concentration around 150 mg/100 ml at the time of delivery Clinical data on mothers and infants are given in Table 1

Gestational diabetic women
Selection criteria for performing an intravenous glucose tolerance test during the last trimester were Family history of diabetes among first degree relatives history of unexplained still birth and neonatal death history of previous large baby (above 4 5 kg) repeated abortions or premature deliveries obesity (weight >90 kg) or glycosuria

An intravenous glucose tolerance test (50 ml of a

The maternal values of FFA and glycerol were significantly related only in controls ($r = 0.68$) and in IGDMs ($r = 0.57$). A significant correlation was found in all groups between the maternal FFA and D β hydroxybutyrate concentrations ($r = 0.76$, 0.64 and 0.47) in the IGDM, IDM and control groups respectively.

Changes in FFA, glycerol, glucose and D β hydroxybutyrate during the first 2 hours

There was a progressive rise in FFA concentrations from 30 minutes and onwards in the IGDM and control groups. As seen in Table 2 the mean values of the control and IGDM groups were similar at all sampling times. In contrast there was only a slight increase in FFA concentrations with time in the IDM group and the mean values were significantly lower than those of the IGDM and control group at all sampling times. The main rise in glycerol concentrations occurred during the first 60 minutes in all groups. The mean values within the groups were similar after 30 minutes (Table 2).

Following birth glucose concentrations dropped significantly in all groups and the lowest mean values were found at 60 minutes. The decrease in glucose concentration was most pronounced in the IDM group and the mean values were significantly lower than in the 2 other groups from 30 minutes and onwards. The mean values of the IGDM and control groups were not statistically different.

All groups had their highest mean value for D β hydroxybutyrate at birth. The IGDM group had the highest initial mean level and all groups showed a significant drop during the first 30 to 60 minutes. Thereafter the values remained low and were essentially unchanged.

Significant correlations between FFA and glycerol concentrations were found at 60, 90 and 120 minutes in the IGDM and control groups, but in the IDM group only at 90 minutes. No correlations were found between FFA or glycerol and glucose concentrations in any group of the infants.

Table 4. *Mean maternal glucose values (mg/100 ml) ± S.D. in IGDM and in subgroups of IDM (White classes) during ten days before delivery*

Time of day	IGDM	IDM White classes B	C	D
08.00	88.3 (12) ±13.4	79.8 (6) ±13.5	98.9 (16) ±22.2	114.4 (12) ±23.1
10.00	111.7 (9) ±20.8	129.7 (5) ±18.4	142.3 (8) ±25.6	160.6 (5) ±50.6
12.00	92.0 (9) ±16.6	82.6 (6) ±17.7	109.4 (16) ±29.9	119.6 (12) ±33
16.00	103.6 (10) ±9.2	108.9 (5) ±9.3	110.7 (16) ±20.9	112.1 (12) ±35.6
19.00	121.1 (10) ±31.1	141.3 (6) ±20.9	144.6 (16) ±31.1	147.5 (12) ±41.3

Influence of pre-natal history

Maternal blood glucose concentrations in the IGDM and IDM groups, the latter subgrouped according to White (37), are summarized in Table 4. Individual mean values were calculated for each of the 5 sampling times of the day during 10 days prior to delivery. Group mean values ± S.D. were calculated on the individual mean values. Significantly higher mean group values were found only for White class D at 08 and 10 hours ($p < 0.01$ and $p < 0.005$) and White class C at 10 hours ($p < 0.05$) as compared with the values of the IGDM group. There was no obvious relation between maternal mean blood glucose concentrations in White classes B, C and D and the infant values of FFA, glycerol, glucose and D β hydroxybutyrate of corresponding White groups. Infant group mean values of FFA, glycerol, glucose and D β-hydroxybutyrate were not significantly different between White classes B, C and D. The postnatal patterns of changes of FFA, glycerol, glucose and D β-hydroxybutyrate were unrelated to the type of delivery.

DISCUSSION

The present findings extend and confirm previous observations of much lower than normal mean plasma concentrations of FFA and glucose in IDMs during the first few hours after birth (5, 10, 11, 12, 25, 34). However

Table 3 *Comparison between umbilical arterial (UA) and umbilical venous (UV) plasma values of FFA glycerol D β hydroxybutyrate and glucose in 3 IDMs during the first 2 hours after birth*

Value	Sampling site	Mean (range)	Number of paired samples	p_t[a]	r[b]	p
FFA mM	UA	0 29 (0 06–1 09)	16	ns	0 99	<0 001
	UV	0 29 (0 09–1 08)				
Glycerol mM	UA	0 202 (0 069–0 532)	11	ns	0 99	<0 001
	UV	0 190 (0 064–0 452)				
D β hydroxy butyrate mM	UA	0 076 (0–0 232)	13	ns	1 00	<0 001
	UV	0 071 (0–0 203)				
Glucose mg/100 ml	UA	53 6 (0–199)	16	ns	0 94	<0 001
	UV	55 9 (0–168)				

[a] p_t – degree of significance between UA and UV values using paired t test
[b] r – correlation coefficient between UA and UV values
[c] p_r – degree of significance of the correlation coefficient r

venous catheters or only venous catheters were inserted The catheters were kept patent by intermittent flushing with 0 15 M sodium chloride solution (maximum amount administered was 15 ml) No heparin was used At delivery blood samples were obtained simultaneously from a maternal cubital vein and an umbilical vein Blood samples (1 5 ml) were taken in heparinized plastic syringes at 30 60 90 and 120 minutes of age

Analysis

Blood samples were placed in chilled plastic tubes and kept on ice Plasma was separated within 1 to 2 hours of the sampling and stored at −20 C until analysed Glycerol (13) FFA (14) *D β* hydroxybutyrate (27) and glucose (15) were determined on plasma All analysis were done in duplicate and the methodological errors were below 5 for glucose FFA glycerol and *D β* hydroxybutyrate

RESULTS

Mean values ± standard deviations of plasma concentrations of FFA glycerol glucose and *D β* hydroxybutyrate in IDM IGDM and control infants during the first 2 hours after birth and in their mothers after delivery are given in Table 2 Mean values of the 7 control infants were not different from those of a previous study (32) and they have therefore been combined Significant differences between mean control values and corresponding mean values of IDM and IGDM groups are given in Table 2 There were no significant differences between paired arterial and venous concentrations of FFA glycerol *D β* hydroxybutyrate and glucose (Table 3) Therefore values determined on umbilical venous blood the only samples obtained in a few subjects have been included in the statistical calculations

Maternal and infant values of FFA glycerol glucose and D β hydroxybutyrate at birth

The maternal glucose concentrations were not significantly different between the groups The maternal values of FFA glycerol and *D β* hydroxybutyrate were significantly higher in the IGDM group as compared with those of the control group and the IDM group The mean FFA concentration in cord blood was similar in the three groups The IDM and IGDM groups had glycerol and glucose concentrations in cord blood which were significantly above the mean control values The concentration of *D β* hydroxybutyrate in cord blood was significantly higher only in the IGDM group as compared with the mean control value In all 3 groups there were highly significant correlations between maternal and umbilical venous blood concentrations of both glucose ($r = 0\ 65$ $r = 0\ 77$ $r = 0\ 73$) and of *D β* hydroxybutyrate ($r = 0\ 98$ $r = 1\ 0$ and $r = 0\ 95$) in the IDM IGDM and control groups respectively A significant relationship between maternal and umbilical venous concentrations of FFA was found only in the IDM group ($r = 0\ 62$) and for glycerol concentrations in the control group ($r = 0\ 99$) and in the IGDM group ($r = 0\ 61$)

The maternal values of FFA and glycerol were significantly related only in controls ($r = 0.68$) and in IGDMs ($r = 0.57$). A significant correlation was found in all groups between the maternal FFA and D β hydroxybutyrate concentrations ($r = 0.76$, 0.64 and 0.47) in the IGDM, IDM and control groups respectively.

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DISCUSSION

The present findings extend and confirm previous observations of much lower than normal mean plasma concentrations of FFA and glucose in IDMs during the first few hours after birth (5, 10, 11, 12, 25, 34). However

IGDMs showed a postnatal increase in FFA values comparable to that in the controls which is in accordance with one previous report (10) but in contrast to others (5 12) An unexpected finding was that the IDMs all showed a similar and significant rise in mean glycerol concentrations irrespective of White classification This rise was not different from that seen in IGDMs and controls, suggesting a comparable increase of lipolysis in all groups of infants studied

Several possible explanations could account for this apparent discrepancy between plasma levels of glycerol and FFA in the IDM group The most likely explanation is a decreased outflow of FFA from adipose tissue because of an increased rate of re esterification of liberated fatty acids with α glycerophosphate derived either from glucose entering the cell or from glycogen stored within the cell (26) A state of functional hyperinsulinism (1) would lead to an accelerated entry of glucose into adipose tissue cells and other tissues which would also explain the low plasma glucose concentrations This interpretation is however difficult to reconcile with the strong antilipolytic effect of insulin In vitro experiments have demonstrated that this effect which is probably due to inhibition of adenyl cyclase (3) also occurs at significantly lower concentration of insulin than that needed to effect the rate of uptake of glucose by adipose tissue (2 8)

Novak et al have recently reported elevated glycogen concentrations of subcutaneous adipose tissue obtained from newborn normal infants at birth (18) The glycogen content of adipose tissue decreased rapidly during the first hours after birth and they suggested that during this period the availability of glycogen would favour an increased re esterification of fatty acids This suggestion is supported by *in vitro* studies of isolated adipose tissue cells from normal newborn infants which showed a high release of glycerol and a low outflow of FFA to the medium (17 18) and indirectly by our previous *in vivo* studies (32) The importance of adipose tissue glycogen as a regulator of FFA release in the immediate neonatal period is further supported by the observations of an inverse correlation between the length of labour and glycogen concentration in newborn infants less that 4 hours of age (18) and of a positive relationship between length of labour and infant arterial FFA concentration at birth (32)

Studies on body composition of IDMs have indirectly suggested increased intracellular carbohydrate stores (9 21) Recently it has also been demonstrated that the glycogen content in subcutaneous adipose tissue of newborn IDMs was higher than in normal infants (19) The prerequisites for an increased rate of re esterification of FFA in adipose tissue of IDMs would therefore seem to be fulfilled

There are other less likely explanations for the discrepancy between the plasma concentrations of glycerol and FFA in IDMs during the first 2 hours after birth One possibility could be that following lipolysis in adipose tissue the liberated fatty acids are released to the blood stream A much increased removal rate of FFA from the blood must then be assumed in order to explain the low plasma FFA concentrations This in turn would imply an increased utilization and/or oxidation of FFA which seems unlikely in view of observed low and unchanged plasma concentrations of *D β* hydroxybutyrate and also in view of observed normal oxygen consumption and RQ values around 0 9 during the first hours after birth (29)

Very low blood glucose concentrations (below 20 mg/100 ml) are frequently seen in IDMs—as in the present study—and occasionally also in apparently healthy normal infants during the first hours after birth without any clinical signs or symptoms of hypoglycemia It has been suggested that substrates other than glucose could be utilized by the brain during the neonatal period Although strong experimental evidence indicate that acetoacetate and *D β* hydroxybutyrate could replace glucose as substrate for the growing brain (22 31) these substrates are more likely to be important

sources of energy later during the neonatal period In agreement with previous studies were the present observations that the concentration of D β hydroxybutyrate in cord blood was dependent on the maternal value at the time of delivery (27) The levels of D β hydroxybutyrate also declined rapidly after birth in all groups and from 30 minutes and onwards the concentrations were below those at which measurable cerebral arterio-venous differences occurred in children aged 6 weeks or more (31)

Although quantitative data are lacking it is quite possible that IDMs have elevated glycogen concentration also in other organs While it is sometimes cited that the neonatal brain contains no more glycogen than the adult brain (7) it has been demonstrated that newborn cats and dogs have 3–4 fold higher glycogen concentrations in the spinal cord and medulla than adult animals (6) It is tempting to suggest an increased availability of glycogen in these and perhaps other parts of the brain also in human newborns and to a greater extent so in IDMs This hypothesis would help to explain why hypoglucosemia is usually tolerated during the first hours after birth in both IDMs and normal infants whereas similarly low blood glucose concentrations at a later age more often are accompanied with the clinical manifestations of hypoglycemia Furth r studies including determination of glycogen concentrations in different parts of the central nervous system of IDMs are of course necessary preliminaries to such speculations

The present findings of hypoglucosemia low plasma concentrations of FFA and increasing levels of plasma glycerol can also in part be explained by functional hyp rinsulinism The relative importance however of increased glycogen stores as compared with that of hyperinsulinism" during the first 2 hours after birth in IDMs is difficult to evaluate from the present data Both increased glycogen stores and hyperinsulinism postnatally are compatible with the concept of maternal hyperglycemia—fetal hyp rinsulinism (23)

It is clear that strict supervision of diabetic pregnancies reduces perinatal mortality rate and normalizes birth weight of the infants (16 20 23 30) These much improved results are probably to a great extent related to the achieved degree of maternal metabolic control (23) It has previously been demonstrated that both the mean maternal glucose level during the last month of pregnancy and the maternal glucose concentration at delivery are related to the infant s mean glucose level during the first 24 hours after birth (23) In the present study there was no apparent relationship between maternal blood glucose values calculated as individual or group mean values and the pattern of changes in infant plasma values of glucose FFA and glycerol This could indicate that five daily maternal glucose values during ten consecutive days before delivery were not enough in order to express the achieved degree of metabolic control with respect to its possible influence on the metabolic adjustment of the newborn infant

SUMMARY

Arterial concentrations of FFA glycerol glucose and D β hydroxybutyrate were serially measured during the first 2 hours after birth in IDMs IGDMs and in control infants All diabetic mothers were subjected to a well defined p ogram of control during pregnancy IDMs had only a slight increase in mean plasma FFA concentrations and the values were significantly lower than those of the IGDM and control groups at all times In contrast the rise in mean plasma glycerol values was significant and similar in all groups suggesting a comparable increase in lipolysis Following birth IDMs showed a more pronounced decrease in mean plasma glucose values than the other groups Mean plasma values of D β hydroxybutyrate showed a significant drop during the first 60 minutes and thereafter the values remained low and were not significantly different between the groups The pattern of changes in FFA glycerol and glucose was not influenced by type of delivery duration of diabetes and/or

presence of retinopathy in the mothers nor was there any apparent relationship to the degree of maternal metabolic control during pregnancy It is suggested that the low mean FFA levels despite of increasing mean glycerol concentrations in IDMs are explained by an increased rate of re esterification of FFA within adipose tissue The e findings can only partly be explained by postnatal functional hyperinsulinism The recent demonstration of higher than normal glycogen concentrations within adipose tissue in IDMs offers a more plausible explanation for the increased rate of re esterification during the first hours after birth The present data do not allow of conclusions as to the relative importance of increased glycogen stores as compared with that of hyperinsulinism during the first 2 hours after birth in IDMs

ACKNOWLEDGEMENTS

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PROPERTIES OF FIVE ACID HYDROLASES IN HUMAN SKIN FIBROBLAST CULTURES

Possible Use in the Diagnosis of Inborn Lysosomal Diseases

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There is now abundant evidence of the importance of cell cultures in the study of inborn errors of metabolism At present there are about 40 genetic errors which have been shown to affect specific molecules in human cultured cells (15 16 24) The mutant phenotypes include abnormalities of carbohydrate amino acid lipid and nucleotide metabolism and among them are extremely serious diseases such as the mucopolysaccharidoses sphingolipidoses and the glycoprotein degradation diseases

Fibroblastic cell lines can now easily be derived from human tissues and these cell lines can be maintained *in vitro* under successive subcultivation for a period of several months This allows repeated biochemical analyses on fresh material and the use of methods impossible to use *in vivo* in order to elucidate and confirm diagnoses of genetic diseases

The purpose of this investigation was to evaluate enzymatic analyses of cultured skin fibroblasts as a diagnostic technique in inborn lysosomal diseases by studying the properties of five relevant hydrolytic enzymes with acid pH optima

MATERIAL AND METHODS

Controls

As controls young adults of both sexes (laboratory personnel) were used and also children hospitalized for minor illnesses apparently not influencing their general health Since no obvious age or sex difference was noted all results were calculated together This does not imply that minor differences could not exist If such differences do exist they would however not influence the conclusions drawn

Patients

Analyses were also made on fibroblast cultures from patients with different diagnoses Diagnoses age and sex are shown in Table 1 For cases 5–17 a very thorough diagnostic study had been made Cases 1–4 in whom no clear diagnosis could be made will be described shortly here

Case 1 Male 7 Mental retardation and stunted growth A somewhat large head short neck and peculiar facies with hypertelorism enlarged tongue and low sitting ears Roentgenographically there are changes in the spinal column reminiscent of those in Hurler's disease vertebral bodies short in the sagittal direction first lumbar vertebra displaced backwards resulting in a dorso-lumbar kyphosis Glycosaminoglycan and oligoglycan excretion in urine was normal

Case 2 Male 10 Severe mental retardation and a constitution reminiscent of that of Marfan's syndrome The boy is tall and slender with long extremities fingers and toes There is lens clouding of both eyes (but no lens ectopia) and a cardiovascular malformation (truncus arteriosus and ventricular septal defect) A constant moderate thrombocytopenia has been noted Glycosaminoglycan excretion in urine was normal

Case 3 Male 6 months with signs of osteopetrosis Roentgenographically there is increased density of the skeleton Hepatosplenomegalia and anemia are present

Case 4 Male 6 Retardation of psychomotoric development from about 3 years of age From about 3 1/2 years of age epileptic convulsions There are signs of progressive spinal muscular atrophia with

Table 1 *Activity of acid hydrolases in cultured fibroblasts*

Activity expressed as U/g protein

Diagnosis	Sex and age	Sub-culture	β galacto-sidase	β glucosidase	α mannosidase	N acetyl β glucosaminidase	β-glucuronidase
Controls (n = 10)		P1 P2	4.80 ± 2.25	0.368 ± 0.153	0.789 ± 0.341	67.0 ± 28.9	0.486 ± 0.228
1 —	M7	P1	0.370	0.320	1.11	74.5	0.56
		P2	0.180	0.843	1.03	120.9	1.69
		P5	0.00	—	—	101.7	—
2 —	M10	P1	6.22	0.311	1.08	73.2	0.301
3 —	M8/12	P1	1.82	0.052	0.193	26.9	0.711
		P2	6.12	0.342	0.778	64.5	0.497
		P6	11.7	0.442	1.43	175	0.952
4 —	M6	P1	4.06	0.196	0.853	56.1	1.052
(new skin biopsy of patient no 4)		P3	10.6	0.331	3.20	195	1.25
5 Hurler's syndrome	M8	P1	3.48	0.135	0.226	28.6	0.243
		P2	4.96	0.231	0.514	53.8	0.374
		P5	6.43	0.364	1.078	70.5	0.563
Mother of case 5		P1	8.82	0.511	1.10	107	0.66
Father of case 5		P1	6.30	0.382	1.12	88	0.503
6 Marfan like syndrome	M8	P2	7.69	0.641	0.869	63.3	0.037
		P3	3.93	0.262	1.20	93.9	0.714
7 Marfan like syndrome	F20	P1	1.21	0.090	1.19	30.9	0.243
8 Cystic fibrosis	M14	P2	5.92	0.582	0.388	86.9	0.946
Mother of case 8		P2	6.76	1.13	0.450	64.8	0.714
Father of case 8		P2	5.25	0.750	1 0	117.5	0.361
9 Acute leukemia	M4	P1	1.65	0.200	0.59	36.6	0.20
10 Diabetes	M9	P1	4.36	0.110	0.79	39.9	0.18
11 Down's syndrome	M1	P1	3.65	0.280	0.87	51.2	0.30
12 Recklinghausen's disease	M14	P4	11.9	0.549	1.39	238	1.52
13 Hyperglycinemia	M1	P2	9.21	0.666	1.11	195	1.16
14 Werdnig Hoffman's disease	M1	P4	6.65	0.284	3.69	101	0.485
15 Gaucher's disease	M6	P1	10.3	0.057	1.63	190	1.04
		P3	14.3	0.013	1.37	229	1.95
Mother of case 15		P2	3.61	0.225	1.83	119	0.381
16 Gaucher's disease	M5	P1	8.4	0.080	1.52	104	0.694
Mother of case 16		P1	1.91	0.341	2.15	200	0.262
17 Sanfilippo's syndrome	M13	P4	12.3	0.490	2.76	278	1.98

Control values given as mean ± standard deviation

proximal muscular weakness. His facial features are coarse and reminiscent of those in Hurler's disease. Glycosaminoglycan and oligoglycan excretion in urine is normal, however.

Skin biopsies

Biopsies were taken from the extensor surface of the upper arm and established in cultures similar to those of other workers (4). Cells were maintained in 30 ml culture bottles (Falcon Plastics, Los Angeles). The medium used was Eagle BME (Flow Labs, Irvine, Scotland) with Hanks salts, 20% newborn calf serum, penicillin 100 IE/ml and streptomycin 100 mg/ml.

Enzymatic analyses

At the stage of confluent monolayer cells were removed by trypsinization (0.25% trypsin solution, Flow Labs), centrifuged at 1 500 g min for 5 min at room temperature, washed twice with saline and then frozen at −20 C until analysis (not more than 2 weeks later). No change of enzyme activity occurred during this time.

Table 2 Kinetic properties of five acid hydrolases in fibroblast homogenates

Enzyme	Actual substrate concentration	pH optimum	K_m (mM)	of V_{max} at actual substrate concentration ( )
β galactosidase	0 65 mM	4 5 (0 16 M acetate buffer)	3 35	20
β glucosidase	0 50 mM	5 0–5 5 (0 05 M acetate or 0 25 M citrate buffer)	0 50	50
α mannosidase	8 85 mM	4 5 (0 14 M citrate buffer)	1 78	78
N acetyl β glucos aminidase	1 87 mM	4 0 (0 25 M citrate buffer)	0 53	85
β glucuronidase	3 33 mM	4 5 (0 25 M acetate buffer)	0 47	80

The cells from a culture bottle were homogenized in 4 ml of aq redest The crude homogenate was used for enzyme activity assays as described earlier (8) Gel filtration (Sephadex G 150 column 70×3 cm Pharmacia Uppsala Sweden) and isoelectric focusing (commercial equipment LKB Produkter Stockholm Sweden) of soluble fibroblast fractions (crude homogenate centrifuged at 100 000 g for 60 min supernatant used as enzyme source) were performed as described elsewhere (8) Ceramide galactosidase was measured as in other tissues (7) 4 Methylumbelliferyl glycosidic substrates (Koch Light Labs Colnbrook Bucks England) were used for β galactosidase (β D galactoside galactohydrolase EC 3 2 1 23) β glucosidase (β D glucoside glucohydrolase EC 3 2 1 21) α mannosidase (α D mannoside mannohydrolase EC 3 2 1 24) p nitrophenyl glycosidic substrate (Koch Light Labs) for N acetyl β glucosaminidase (β 2 acetylamino 2-deoxy D glucoside acetylaminodeoxy glucohydrolase EC 3 2 1 30) and phenolphthalein glycosidic substrate (Koch Light Labs) for β glucuronidase (β D-glucuronide glucuronohydrolase EC 3 2 1 31) Ceramide β galactoside (N lignoceroyl DL sfingosyl β D galactoside kerasin) was obtained from Seravac Lab Maidenhead England

Common to all methods was that the pH of the incubation mixture was the pH optimum for the enzyme with the buffer and substrate used The concentration of the substrate was not always optimal the solubility of some substrates not being large enough (see Results kinetic properties) In all methods the enzyme activity measured was directly proportional to the amount of tissue in the incubation tube Non enzymatic hydrolysis of the substrates used was negligible

Protein was assayed according to Lowry et al (1_)

RESULTS

Kinetic properties

In Table 2 are shown the substrate concentrations obtained with the synthetic substrates used pH optimum K_m and of V_{max} at the actual substrate concentrations The high K_m observed for β galactosidase and β glucosidase indicates inhibition of the enzyme in fibroblast homogenate A purified β galactosidase preparation gives a K value of 2×10^{-4} M with the same buffer and at the same pH while purified β glucosidase exhibits a K_m of 5×10 M (unpublished observations)

Molecular forms

Isoelectric focusing of the soluble fraction of fibroblast homogenates (Fig 1) showed dominance of high molecular weight forms of β galactosidase with pI 4 2–4 8 β glucosidase activity was found around pH 4 5 and at pH 3 9–4 2 probably high molecular forms of th enzyme

The other enzymes investigated exhibited the same type of activities as earlier described for liver tissue (8) With gel filtration (Fig 2) enzyme patterns similar to those of liver tissue (8) were obtained with the exception that as with isoelectric focusing high molecular forms of β galactosidase and β glucosidase were found

Enzyme activity and cell culture age

At the beginning of cell cultivation the lysosomal enzymes investigated here showed low activities All enzyme activities then increased with increasing age of the culture From the first subculture to the second (2nd to 3rd week) there was a 1 5–3 fold increase in activity for all enzymes investigated and from the first to the third (3rd to 4th week) there was a 3 5–7 fold increase

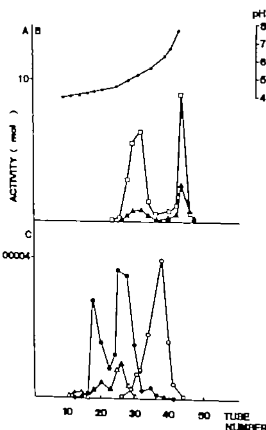


Fig 1 Isoelectric focusing of the soluble fraction from a fibroblast homogenate An amount containing 2 mg of protein was applied to the column □—□ N acetyl β glucosaminidase ▲—▲ β glucuronidase ●—● β galactosidase △—△ β glucosidase ○—○ mannosidase Activity given as nmoles substrate cleaved per minute under the above mentioned conditions Scale A refers to hexosaminidase activity and B to β glucuronidase activity Scale C is valid for the three other enzymes

Findings in patients

Case 1 showed a marked decrease of β galactosidase activity in several subcultures (P_1 P P) Other patients as expected sometimes showed low enzyme activities in the first sub culture but the activity was then normalized with the time of cultivation

In the patients with Gaucher s disease β-glucosidase activity was repeatedly low while the other enzymes exhibited high activities Cells from the patient with cystic fibrosis had normal activities of all enzymes including β glucuronidase Cells from the patients with Hurler s disease and Sanfilippo s disease had normal activities of the enzymes investigated including β galactosidase

In Table 3 are shown the findings when the natural substrate ceramide galactoside was used in the assay of β galactosidase activity There was a somewhat low but definite activity towards this substrate in fibroblast homogenates from the patient with Hurler s disease

DISCUSSION

Our values of enzyme activities in cultured fibroblasts are in good agreement with those of other authors (1 2 11)

The level of activity of the enzymes inves

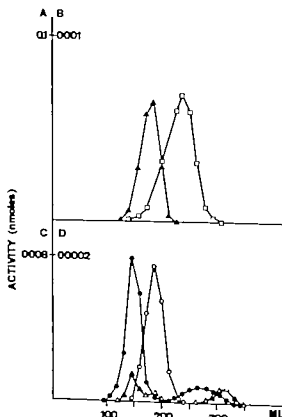


Fig 2 Gel filtration on Sephadex G 150 of the soluble fraction from a fibroblast homogenate An amount containing 4 mg of protein was applied to the column Caption as in fig 1 Activity given as nmoles substrate cleaved per minute Scale A refers to hexosaminidase activity scale B to β glucuronidase scale C to α mannosidase and β galactosidase activity and scale D to β glucosidase activity

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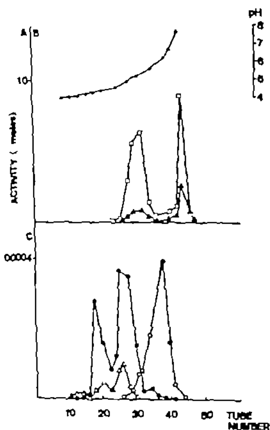


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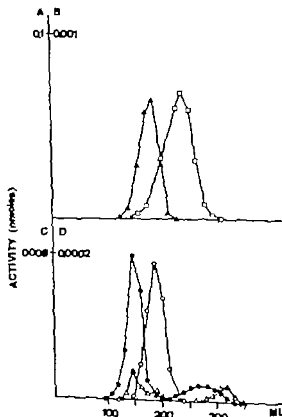


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Table 3 *Activity of ceramide galactoside in cultured human fibroblasts*

Activity expressed as U/g protein

	Activity
Control	
1	0 12
2	0 10
3	0 32
5 Hurler's syndrome	0 06

tigated here is different in fibroblast homogenates and liver tissue homogenates (Table 4) The enzyme activities are higher in liver homogenates except for β galactosidase where we found the same activity level in both types of homogenates and *N* acetyl β glucosaminidase which in fibroblast homogenates exhibits twice the activity of liver homogenates when corrected for using citrate buffer instead of acetate buffer as in earlier investigations (In the assay of *N* acetyl β glucosaminidase citrate buffer pH 4 0 gives about twice the activity obtained with acetate buffer of the same pH)

Compared to the enzyme activity levels in untreated skin biopsies those of cultured skin fibroblast homogenates are higher (Table 4) The enzyme pattern of the fibroblast homogenate is however similar to that of skin biopsies with *N* acetyl β glucosaminidase as highest activity and β glucosidase as lowest and the three other enzymes with intermediary activities

pH dependency of the enzyme activities in fibroblast cultures is similar but not totally identical with the enzyme activities earlier found in liver homogenates (5 6 17 21 23)

β galactosidase and β glucosidase can under certain conditions of ionic strength and pH form high molecular weight aggregates with lower isoelectric points than the smaller molecule activities (8) This can explain the difference between the findings in fibroblast homogenates and liver homogenates In conclusion therefore the isoenzyme pattern and pH dependency type of the enzymes indicate that all five enzymes in fibroblast cultures are very similar to those found in liver preparations It

Table 4 *Mean activity of acid hydrolases in liver biopsy skin biopsy and fibroblast homogenate*

Enzyme	Liver biopsy	Skin biopsy	Fibroblast P1–P2
β galactosidase	4 79[a]	0 39[a]	4 80
β glucosidase	0 63[b]	0 08[b]	0 37
α mannosidase	5 18[a]	0 62[b]	0 79
N acetyl β glucosaminidase	13 3	2 68[a]	67 0
β glucuronidase	4 32	0 18[c]	0 49

Mean given as U/g protein
[a] From Lundquist & Öckerman (13)
[b] Own unpublished findings Four biopsies are used and mean is calculated
[c] From Öckerman (22)

should thus be possible to use skin fibroblast cultures to detect and confirm lysosomal storage disorders caused by a deficiency of one of these enzymes This has in fact also been done in Tay Sachs disease (*N* acetyl β glucosaminidase isoenzyme A) (20) GM_1 gangliosidosis (β galactosidase) (25) and in Gaucher's disease (β glucosidase) (2) Our findings in the patient with Gaucher's disease are in good agreement with those of Beutler et al (2) A fibroblast deficiency of β glucuronidase in a patient with an atypical mucopolysaccharidosis has recently been described by Neufeld et al (18) In I cell disease several of these enzymes have decreased activities (11)

When fibroblast cultures are used for clinical purposes to detect an enzyme deficiency it is important to assay the enzyme in several successive subcultures A low enzyme activity in an early subculture may be normalized in a later one indicating not an enzymatic defect but the influence of the culture conditions Lysosomal enzyme activities so far studied increase with increasing age of the cell cultures (15) This points out possible difficulties of using cell cultures in diagnosis of heterozygous conditions A genetic enzyme defect is however expected to give constantly decreased activities independent of cell culture age as also illustrated by the cases in this work

A thoroughly standardized technique is thus necessary when cell cultures are used in diag

nosis of inborn errors of metabolism The results must be judged from experience of the influence of the culture conditions used The age and density of the culture and also the cell type must be taken into consideration

As already noted β galactosidase activity in skin fibroblast culture seems not in contrast to several other tissues to be significantly decreased in mucopolysaccharidosis types I III (1) The high K_m of this enzyme in fibroblast homogenates indicates however inhibition of the enzyme and the method is therefore not optimal There is no decrease in β galactosidase activity in skin fibroblasts from patients with mucopolysaccharidosis not even after several months of cultivation and when metachromasia occurs This does not indicate inhibition of the β galactosidase activity by the mucopolysaccharides stored an explanation which has been put forward to explain the decreased β-galactosidase activity in other tissues Neither do our findings with a natural β galactosidic substrate indicate a markedly decreased β-galactosidase activity in skin fibroblasts from patients with Hurler s disease The enzymatic defect of Hurler s (and Scheie s) disease has recently been reported by Matalon & Dorfman (14) as an α iduronidase deficiency present in cultured skin fibroblasts Kresse & Neufeld (10) have shown that the enzyme functionally missing in Sanfilippo s disease A is a heparansulphate sulphatase and O Brien (19) has demonstrated that the other form Sanfilippo s disease B has a deficiency of *N* acetyl-α glucosaminidase

Case 1 with decreased β-galactosidase activity in several subcultures has rather high activities of the other lysosomal enzymes This phenomenon has been noticed before in lysosomal disease With our present knowledge it is not possible to classify this patient He may illustrate the difficulties sometimes obtained when correlating glycosaminoglycan storage and excretion enzyme activities and clinical symptoms and signs

A low activity of β-glucuronidase has been reported in sweat glands from patients with cystic fibrosis (3) This decrease could not be demonstrated in cultivated fibroblasts (9) and our results in case 8 are in agreement with this latter finding

SUMMARY

Five acid hydrolases β galactosidase β glucosidase α mannosidase *N* acetyl β glucosaminidase and β glucuronidase were studied in human skin fibroblast cultures The pH-dependency and kinetic properties of the enzymes as well as results of gel filtration and isoelectric focusing indicate that these enzymes are the same in fibroblasts as in liver

Techniques were defined with the aim of finding methods suitable for the diagnosis of inborn lysosomal diseases It is stressed that great caution must be executed in the interpretation of results since isolated enzyme deficiencies may appear in early but not in later subcultures Also there were great variations in the activity levels between different subcultures

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EXCESS GLYCOSAMINOGLYCAN EXCRETION IN INFANCY AND CHILDHOOD

C. A. PENNOCK, FRANCES WHITE, D. MURPHY, R. G. CHARLES and HELEN KERR

From the Department of Chemical Pathology, United Bristol Hospitals, Bristol, U.K.

Increased excretion of glycosaminoglycans (GAG) formerly called mucopolysaccharides is a feature of the mucopolysaccharidoses (10) and examination of urinary GAG is not only regarded as a useful diagnostic procedure but also the disorders have been classified according to the type of GAG excreted (7).

The structure and metabolism of GAG has been reviewed by Muir (11). Briefly these substances are macromolecules consisting of repeating units of an N acetylated hexosamine and an hexuronic acid such that the molar ratio of hexosamine to hexuronic acid is close to unity (except in keratan sulphate in which galactose replaces the hexuronic acid moiety). There are differences in the hexosamine and/or hexuronic acid in the different GAG. The GAG normally excreted in urine is a mixture of chondroitin sulphates which, like hyaluronic acid, are digested by testicular hyaluronidase.

In the mucopolysaccharidoses the major GAG excreted in excess are heparan sulphate, dermatan sulphate and keratan sulphate in differing proportions depending on the disease type (7). These three are all resistant to testicular hyaluronidase digestion and only found in trace amounts in normal urine.

A number of simple screening tests have been devised for detection of excess GAG excretion and their relative merits have been discussed (14). A method based on measuring the turbidity produced when cetylpyridinium chloride is added to buffered urine is thought to be best as it is roughly quantitative and can be related to creatinine excretion to overcome the problems of incomplete 24 hour urine collection (13). It can be applied to random samples of urine, especially useful in infancy when 24 hour collections are most difficult to obtain. It has already been shown that the problems of incomplete collection can be overcome by calculating the GAG/creatinine ratio (17). GAG excretion relative to creatinine varies with age and is highest in the first year of life reaching a peak in the third week (15) so that abnormal high GAG excretion typical of the mucopolysaccharidoses may be difficult to detect at this age.

Urine GAG can be isolated by formation of a macromolecular complex with quaternary ammonium compounds (4) and a positive screening test may be investigated further by estimation of the hexuronic acid content of this complex, except in the case of keratan sulphate which does not contain hexuronic acid and is excreted in excess in Morquio's disease (Type IV mucopolysaccharidosis). Methods for further analysis are usually elaborate and time-consuming. We have adopted a simplified approach relying on confirmation of excess excretion, assessment of resistance to testicular hyaluronidase digestion and analysis of hexosamines present to identify the mucopolysaccharidosis on the basis of Kap-

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lan s classification (7) We report here our experience using this approach to samples received from infants and children with mental and physical retardation

MATERIALS AND METHODS

Random samples of urine were received from approximately 3 000 children of whom 800 were infants below one year of age who were being screened for possible metabolic disorders

Screening for excess GAG excretion was done using a cetylpyridinium chloride (CPC) turbidity test (13) Results were expressed as CPC units per g creatinine where one CPC unit is equal to the turbidity of a solution containing 1 mg chondroitin sulphate per 100 ml of water under the conditions of the test The upper limit of normal was taken as 300 units per g creatinine in the first year of life and from the original data (13) in older children Denny and Dutton s modification (3) of Dorfman s (5) acid albumin screening test for excess GAG excretion was also done on samples from patients with suspected bone dysplasias Creatinine was estimated using an auto analyser method (Technicon method fil N 11)

Samples giving a positive CPC screening test were analysed in more detail The GAG were precipitated from 5 ml of urine by the method of Diferrante (4) using CPC as precipitant and the hexuronic acid content of the precipitate measured by the method of Bitter & Muir (1) Results were expressed as mg hexuronic acid per g creatinine and compared with the normal range defined for age (15 16)

When sufficient sample was available the GAG were isolated from the whole sample using CPC as *precipitant and analysis of hexosamines assessment* of resistance to testicular hyaluronidase and electrophoresis were done

A solution of GAG containing a known amount of hexuronic acid (usually between 2 10 mg per 100 ml of water) was used for hexosamine analysis After hydrolysis in 6N hydrochloric acid for 2 hours at 100 C the hydrolysate was dried at 40 C under a stream of nitrogen redissolved in water and dried again twice to remove final traces of acid The hexosamines were converted to their alditol acetates and estimated by gas liquid chromatography (12) The hexuronic acid content of the solution was estimated before hydrolysis and the results expressed as hexosamine/hexuronic acid ratio and the relative proportions of glucosamine (GluN) and galactosamine (GalN) expressed as a percentage of total hexosamine (HexN)

The same solution of isolated GAG was digested with an equal volume of bovine testicular hyaluronidase (British Drug Houses) 1 mg per ml in 0 1M *sodium chloride* acetate buffer (pH 5 6) in 0 15 *M sodium chloride for* 24 hours at 37 C A control without enzyme was incubated in the same buffer for the same time *After* incubation one volume of *CPC citrate* reagent as used in the screening test was added and the turbidity measured at 450 nm after standing at room temperature for 30 minutes Results were expressed as post digestion *turbidity* as a percentage of the control (Hyal R)

Electrophoresis was performed on cellulose acetate *membrane in Michaelis veronal buffer pH 9 2 at 300* volts (constant voltage) for 100 minutes (8) Strips were stained with 1 w/v alcian blue in 2 v/v acetic acid for 30 minutes then washed in running water until a clear background was obtained dried cleared in Whitmor 120 transparency oil (Shandon Scientific Co Ltd) and scanned using a Densicord Photovoltmeter (Photovolt Corporation N Y) This method separates hyaluronic acid heparan sulphate chondroitin sulphates and heparin but dermatan sulphate migrates with the chondroitin sulphates and keratan sulphate migrates with heparan sulphate (9)

RESULTS

Incidence of positive screening tests

Forty two samples out of 800 obtained from infants gave a positive screening test (approximately 5% of samples in this age group) In children above one year of age 85 samples out of approximately 2 200 gave a positive screening test (approximately 2 6% of samples in this age group)

Twenty seven samples out of the total of 127 giving a positive screening test were from patients with bone dysplasias The remaining positive results were obtained on samples from infants and children with a wide variety of clinical diagnoses (see Table 1)

Patients without bone dysplasias

We were unable to obtain further samples *from 10 infants* and *8 children* without bone dysplasias whose sample gave a positive screening test However in all other patients in this group a further sample was obtained and gave a negative screening test when the child had recovered or was no longer receiving drugs (A small group of 11 mentally retarded children who were not on drugs gave negative screening tests on later samples)

CPC precipitable uronic acid was estimated in all positive samples in this group of patients In 14 out of 37 samples from infants and 8 out of 63 samples from children the result was above the upper limit *for the patient s age*

Table 1 *Number of samples giving a positive screening test and raised uronic acid creatinine ratio with the associated diagnosis*

Diagnosis	Infants <1 year		Children >1 year	
	Screening positive	UA/C ratio increased	Screening positive	UA/C ratio increased
Group A				
Suspected bone dysplasias	5	2	22	18
Group B Cause unknown and repeat screening negative				
Mental retardation without bone abnormalities	0	0	11	0
Group C Later screening negative when recovered or drugs withdrawn				
Respiratory infection on Ampicillin	3	0	6	0
Urinary tract infection	5	1	7	0
Jaundice	4	1	0	0
Convulsions On phenobarbitone	1	1	20	3
Convulsions On Phenytoin	0	0	3	0
Coeliac disease	3	0	7	0
Hiatus hernia	1	0	0	0
Temporary Lactose Intolerance	4	3	0	0
Transient Idiopathic Hypercalcaemia	2	2	0	0
Familial odd facies Poor weight gain Nutritional problem only	1	0	0	0
Multiple fractures	1	1	1	1
Group D Further samples not available				
Mongolism	1	1	0	0
Hereditary fructose intolerance	1	1	1	1
Type I Glycogen storage disease	0	0	1	1
Odd facies (Familial)	1	1	0	0
Deafness (Familial)	0	0	2	0
Hyperarrythemia	3	1	0	0
Dystrophia myotonica	1	0	1	0
Bilateral cortical atrophy	1	1	0	0
Episodic vertigo Cause unknown	0	0	1	1
Werdnig Hoffman Disease	0	0	1	1
Hypothyroidism	1	0	0	0
Constrictive pericarditis	1	0	0	0
Pulmonary tuberculosis on therapy	0	0	1	0

(Table 1) In the majority of samples the result was normal indicating that the positive screening test was not due to excess GAG excretion. Sufficient sample was obtained from some patients in this group for further analysis results of which are shown in Table 2.

Two samples were obtained from a child (No 109) with convulsions while receiving phenobarbitone and when not on drugs respectively. The UA/C ratio and qualitative analyses were normal in both samples and there was no significant change in results except that the CPC screening test was negative when the child was not on drugs.

All samples (except numbers 31, 52 and 124) showed similar qualitative results with only a small percentage of isolated material resistant to testicular hyaluronidase, a Hex/UA ratio close to unity and galactosamine as principle hexosamine. These results are consistent with excretion of mainly chondroitin sulphates which was confirmed by electrophoresis. Thus excretion of GAG although increased in some patients was of a normal pattern. Further samples from the exceptional cases have not been made available to us.

Patients with suspected bone dysplasias

(*a*) *Infants*. Five infants had clinical features suggesting a bone dysplasia. There was insuffi

lan s classification (7) We report here our experience using this approach to samples re ceived from infants and children with mental and phy cal retardation

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...tant to ...uronidase	Major bands on electrophoresis
	CS
	(30 HS 55 CS 15 Hep)
	CS (split band)
	CS
...fficient material isolated	
	CS
	CS
	CS
	CS
	CS
	Glycoprotein + CS
	CS
	Insufficient sample
	Insufficient sample
)	Insufficient sample
	CS

the rest of the material migrated as CS (which would include dermatan sulphate (DS) in the buffer used)

Patient No 89 had a spastic quadriplegia associated with poor vision and marked developmental retardation There were early radiological changes of Hurler syndrome in the lumbar spine and pelvis No metachromatic inclusions were seen in peripheral blood lymphocytes or in cultured skin fibroblasts Leucocyte β-galacto idase activity (estimated by Dr Rosemary Stephens) was within normal limits

The urine sample showed a negative acid albumin test and a UA/C ratio just at the upper limit of normal for her age The proportion of hyaluronidase resistant material was high but electrophoresis showed a heavy glycoprotein band and a band migrating as CS (includes DS in buffer used)

Patient No 106 is a female Jamaican infant who was 2 months old when she presented with coarse features a large protuberant tonque marked lethargy and bilateral optic atrophy Although she looked hypothyroid serum thyroxine was normal Other investigations including Wasserman reaction showed no abnormality There were no metachromatic granules in peripheral blood lymphocytes and leucocyte β galactosidase (estimated by Dr Rosemary Stephens) was normal Radiological examination showed periosteal new bone formation in both femora and no other abnormalities A urine sample examined at this time gave a negative screening test and was not examined further At 7 months of age her urine gave a positive CPC screening test and positive acid albumin test The UA/C ratio was raised (60) and the proportion of isolated GAG resistant to hyaluronidase was abnormally high Electrophoresis showed a slow moving fraction of the same mobility as hyaluronic acid HA (30 6) and bands in the position of HS and CS (includes DS) At 8 months a further sam

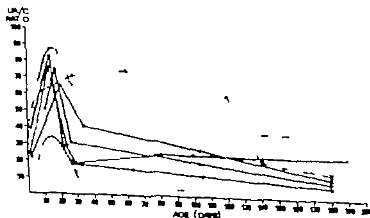


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Table 2 *Results of more detailed analysis on samples from infants and children without bone dysplasia*
Parentheses denote abnormal results — - not done HS - Heparan Sulphate CS - Chondroitin sulphates (*includes* Dermatan sulphate in buffer used) Hep - Heparin

Ref No	Diagnosis	Sex	Age	UA/C ratio	GluNH$_2$	GalNH$_2$	Hex/UA ratio
10[b]	Dystrophia myotonica Transient hypercalcaemia	F	4m	38 0	—	—	—
31[c]	Retarded Cause unknown	F	2y	12 3	42 0	58 0	0 980
40[b]	Hypothyroidism	F	1y5m	21 0	40 1	59 9	1 079
42[c]	Temporary Lactose Intolerance	M	3m	35 3	33 5	76 5	0 970
52[b]	Type I Glycogen storage disease	F	11y	(38 4)	48 6	51 4	(0 24)
66[c]	Familial odd facies Poor weight gain — nutritional problem	M	6m	33 4	17 2	82 8	1 000
101[c]	Urinary tract infection	M	5y	20 6	26 0	74 0	1 143
102	Coeliac disease	M	1y7m	31 7	32 5	67 5	1 072
109a	Convulsions On phenobarbitone	M	6y	13 3	36 7	63 3	0 985
109b[c]	Convulsions No drugs Screening negative	M	6y	17 7	33 5	66 5	0 803
112[c]	Convulsions On phenobarbitone	M	2y10m	16 8	48 0	52 0	1 180
114[c]	Retarded speech and locomotor development	M	2y2m	17 1	21 6	78 4	1 046
116	Convulsions On phenobarbitone	F	4y	(40 0)	25 4	74 6	0 774
117[b]	Bilateral cortical atrophy	F	5m	(104 0)	37 0	63 0	1 300
124[b]	Werdnig Hoffman disease	F	1y6m	(46 8)	(68 3)	(31 7)	0 985
133[c]	Coeliac disease	M	1y8m	21 8	32 2	67 8	0 850

Electrophoresis in veronal buffer pH 9 2
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cient sample for complete analysis from one patient with nutritional rickets (No 54) but the UA/C ratio was raised

Another patient with odd facies multiple skeletal abnormalities and hepatosplenomegaly but without any radiological features of a mucopolysaccharidosis gave the following results A urine sample examined at one month of age gave a positive CPC screening test negative acid albumin test and raised UA/C ratio Qualitative analysis was within normal limits Random samples collected at other times showed a progressive fall in the UA/C ratio *into the normal range* (see Fig 1) associated with a negative screening test by 6 months of age

The other infants were suspected of having Hurler s syndrome and the results of analysis are shown at the beginning of Table 3 Further samples from these infants have not been made available to us

Patient No 43 presented with delayed development odd facies and a large protuberant tongue There was difficulty in abducting the legs and a marked lumbar lordosis The rest *of the physical examination including retino*scopy was normal A number of laboratory investigations including serum thyroxine were normal except for a raised serum alkaline phosphatase (56 and 77 K A units/100 ml on two separate occasions) Metachromatic granules were present in peripheral blood lymphocytes Radiological examination showed abnormal acetabular angles and abnormalities of the vertebral bodies consistent with the early changes of Hurler syndrome (Type I mucopolysaccharidosis) She subsequently died from bronchopneumonia and post mortem examination was refused

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	CS
	(30 HS 55 CS 15 Hep)
	CS (split band)
	CS
...fficient material isolated	
	CS
	CS
	CS
	CS
	CS
	Glycoprotein + CS
	CS
	Insufficient sample
	Insufficient sample
)	Insufficient sample
	CS

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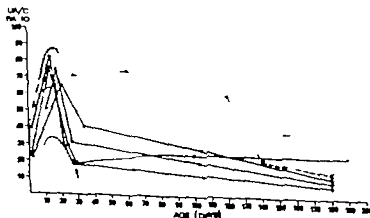


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7)	(30 HS 55 CS 15% Hep)
3	CS (split band)
5	CS
...fficient material	isolated
5	CS
6	CS
3	CS
6	CS
5	CS
7	Glycoprotein + CS
7	CS
5	Insufficient sample
14	Insufficient sample
4)	Insufficient sample
5	CS

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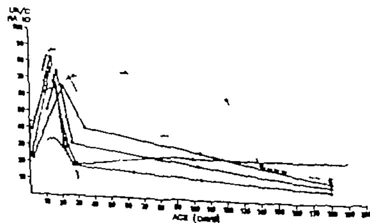


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Table 3 *Results of more detailed analysis on samples from patients with suspected mucopolysaccharidosis*

Parentheses denote abnormal result HA – Hyaluronic acid HS – Heparan sulphate CS – Chondroitin sulphate (includes Dermatan sulphate in buffer used for electrophoresis)

Ref No	Sex	Age	CPC screen	Acid albumin screen	UA/C ratio	GluN	GalN	Hex/UA ratio	Hyal R	Electro-phoretic[a] pattern
43	F	8m	+	Neg	31	(54 2)	(45 8)	(1 427)	(56)	27 HS 73 CS
89	F	7m	+	Neg	38	(56 5)	(43 5)	1 171	(54)	30 Glyco-protein 70 CS
106	F	7m	+	+	(152)	(57 5)	(42 5)	0 745	(62)	31 HA 20 HS 49 CS
1	F	13m	+	+	(183)	25 0	75 0	0 924	(89)	15 HS 85 CS
80	M	14m	+	+	(104)	15 5	84 5	0 852	(100)	18 HS 82 CS (split)
111	F	17m	+	+	(254)	29 5	70 5	0 980	(100)	HS CS Poor separation
4	F	7y	+	+	(119)	(76 9)	(23 1)	0 757	(100)	HS CS Poor separation
59	M	9y	+	Weak +	(76)	(83 3)	(16 7)	0 900	(100)	HS CS
70	M	2y8m	+	+	(60)	(67 0)	(33 0)	0 805	(78)	57 HS (split) 43 CS
84	M	12y10m	+	Neg	(104)	(78 8)	(21 2)	0 880	(100)	HS CS
2	M	15y	+	Weak +	(50)	(100)	—	0 980	(100)	HS only
91	M	11y	+	+	(90)	29 6	70 4	0 935	(100)	31 HS split 69 CS
92	M	8y	+	+	(74)	26 2	73 8	0 860	(100)	22 HS 78 CS

[a] Electrophoresis in veronal buffer pH 9 2
[b] Based on classification of Kaplan 1969 (see text)

ple gave similar qualitative analysis but the UA/C ratio was now higher (152) No new radiological features were seen

(*b*) *Children* Twenty two children were suspected of having a bone dysplasia of which 18 had a raised UA/C ratio

Four patients with bone dysplasias (Marfan s syndrome chondrodysplasia punctata, Cornelia de Lange syndrome and Spielmeyer Vogt syndrome) showed a raised UA/C ratio but qualitative examination of isolated GAG gave normal results Two other patients (Cornelia de Lange syndrome and spondyloepiphyseal dysplasia) had a normal UA/C ratio and there was insufficient sample for further analysis

A further six patients had skeletal abnorma

...terpretation[b] qualitative ...AG analysis	Diagnosis based on clinical and radiological diagnosis
...bnormal but signifi...ance uncertain	Hurler syndrome (MPS Type I)
...bnormal but signifi...ance uncertain	Early changes of Hurler (MPS Type I)
...bnormal but signifi...ance uncertain	Hurler (MPS Type I) or GM gangliosidosis
...bnormal consistent with MPS Type I or V	Hurler (MPS Type I)
...bnormal consistent with MPS Type I or V	Hurler (MPS Type I)
Abnormal consistent with MPS Type I or V	Hurler (MPS Type I)
Abnormal consistent with MPS Type III	Gargoylism
Abnormal consistent with MPS Type III	Sanfilippo syndrome MPS Type III
Abnormal consistent with MPS Type III	Gargoylism
Abnormal consistent with MPS Type III	Sanfilippo syndrome
Abnormal consistent with MPS Type III	MPS Type III
Abnormal consistent with MPS I or V	Scheie's syndrome MPS Type V
Abnormal consistent with MPS I or V	Scheie's syndrome MPS Type V

lities associated with mental retardation for which no cause was found and no definite diagnosis made. The UA/C ratio was raised in 4 and in all of the samples qualitative examination showed a normal pattern with low HyalR and GalN as principle hexosamine. Further samples were not made available to us therefore these cases will not be discussed further.

Ten patients had clinical features of one of the mucopolysaccharidoses and their results are shown in Table 3.

Three children (Nos 1, 80, 111) had typical clinical and radiological features of Hurler syndrome (Type I mucopolysaccharidosis) and all samples gave a positive CPC screening test, a positive acid albumin test and raised UA/C ratio. All of the isolated GAG was resistant to hyaluronidase digestion in two cases and 89 was resistant in the third. The Hex/UA ratio was normal in all three, the principal HexN was GalN and a heavy band of HS was seen on electrophoresis although the predominant band was CS (includes DS in buffer used).

A further 5 children (Nos 4, 59, 70, 84, 2) had some of the features of Hurler syndrome but with less marked radiological changes. Three of them had clear clinical and radiological features of Sanfilippo syndrome (Type III mucopolysaccharidosis) and the other two were called simply Gargoylism. The urine received from all these children gave an abnormal CPC screening test and raised UA/C ratio but only two had a positive acid albumin test. The results of further analysis revealed a similar pattern in all five, a pattern which differed from the Hurler syndrome in that GluN was the principle HexN and HS was the principal band on electrophoresis. All of the isolated material was resistant to hyaluronidase digestion in four cases and 78 resistant in the fifth.

Two brothers (Nos 91, 92) showed typical clinical and radiological features of Scheie's syndrome (Type V mucopolysaccharidosis). The urines gave positive CPC screening tests, positive acid albumin tests and raised UA/C ratios. The isolated GAG was similar to the Hurler syndrome in that all of the material was resistant to hyaluronidase. GalN was the principal HexN and electrophoresis showed CS and HS with the former predominant.

DISCUSSION

The methods used in this study have been simplified for use in a busy routine clinical

chemistry laboratory Their simplicity may have disadvantages in certain unusual cases but our experience suggests that this simplified approach has at least been adequate in the diagnosis of the mucopolysaccharidoses We have not used dialysis in preliminary preparation of samples since low molecular weight GAG may be lost in this way (2) We have not used any column chromatographic procedures as these are time consuming and result in a large number of aliquots for analysis

The merits of the CPC citrate screening test have been discussed elsewhere (14) and the present larger series of results confirms its usefulness It was always positive in children with mucopolysaccharidoses even when Denny and Dutton's modification (3) of Dorfman's acid albumin screening test (5) was negative The latter test does not take urine concentration into account whereas the CPC citrate test does and can be used on random urine samples The CPC citrate test was also positive in a number of other bone dysplasias However false positive results occur notably in children receiving antibiotics and anticonvulsants and infants with various types of malabsorption A higher proportion of positive results is found in infancy probably due to the fact that we keep the normal upper limit of the test as low as possible in order to ensure detection of genuine disorders of GAG excretion GAG excretion is very high relative to creatinine in the first few weeks of life (15) but many positive results at this age become negative at a later date Repeated screening may be necessary in infancy not only to exclude false positives but also to detect those mucopolysaccharidoses which may not have abnormal GAG excretion shortly after birth but may develop it later (vide infra) In our hands the acid albumin test was less reliable for detection of mucopolysaccharidoses being negative in two infants with suspected mucopolysaccharidoses and negative or only weakly positive in older children with Sanfilippo syndrome

Normal children and infants excrete chondroitin sulphate A and C as principal GAG These are digested by testicular hyaluronidase and the principal HexN is GalN In the mucopolysaccharidoses other GAG are excreted in excess all of which are resistant to hyaluronidase digestion notably DS with GalN as principal HexN and HS and KS with GluN as principal hexosamine (7) With the exception of KS all these GAG contain hexuronic acids in the molecule and have a HexN/UA ratio approximating to unity Thus a positive CPC citrate test can be confirmed by the demonstration of a raised CPC precipitable hexuronic acid except in the case of Morquio's disease in which KS is excreted in excess (7) However in this case the screening test will still be positive the isolated material resistant to hyaluronidase digestion the HexN/UA ratio very high and the principal HexN will be GluN results very different from a normal excretion pattern

Electrophoresis may be used as confirmatory analysis but it should be noted that DS migrates with the chondroitin sulphate in barbitone buffer and KS probably migrates with HS (9) We have recently used zinc sulphate as electrolyte medium in which DS separates from CS and KS from HS (6) So far we have confirmed the presence of DS in three patients (Nos 106 111 59 in Table 3) However although separation from CS was good that from HS was poor It is possible that both electrophoretic systems may be useful together but care is still necessary in interpretation especially since urinary material may be under sulphated and thus migrate more slowly than marker samples derived from tissues One advantage is detection of excess glycoprotein which may be coprecipitated with GAG when CPC is used as precipitant The presence of glycoprotein will account for a high HyalR and higher GluN which may be difficult to interpret unless the presence of glycoproteins is confirmed

The expected results by the methods which we have used are summarised in Table 4 which is based on the work of Kaplan (7)

In this laboratory analysis of samples from

Table 4 *Expected results by methods used in this work (based on Kaplan 1969)*

HS = Heparan sulphate which may include keratan sulphate in buffer (see text) CS = Chondroitin sulphates which includes dermatan sulphate in buffer used Where the Hyal R is high it suggests that the CS band is largely dermatan sulphate

Diagnosis	CPC extrato Screening test	UA/C ratio	HexN/UA ratio	Principle HexN	Hyal R	Principle bands on electro-phoresis
Normal child	Neg	Normal	Normal 0 77–1 23	GalN	< 30	CS
I Hurler syndrome	+	High	Normal	GalN	> 80	CS > HS
II Hunter syndrome	+	High	Normal	GluN = GalN	> 80	CS = HS
III Sanfilippo syndrome	+	High	Normal	GluN	> 80	CS < HS
IV Morquio s disease	+	Normal or high	High	GluN	> 80	HS*
V Scheie s syndrome	+	High	Normal	GalN	> 80	CS > HS
VI Maroteaux Lamy syndrome	+	High	Normal	GalN	> 80	CS*
Marfan s syndrome	Neg or +	Normal or high	Normal	GalN	< 30	CS

Veronal buffer pH 9 2.

normal infants and children has shown less than 30 of isolated GAG to be resistant to testicular hyaluronidase digestion The Hex/UA ratio is normally between 0 77 to 1 23 and the principal HexN is GalN the amount of which falls slightly with age although the percentage GalN remains above 60 except in rare instances when heavy glycoprotein excretion is also present (unpublished data)

Examination of Table 2 shows that when sufficient sample was available the majority of children giving a false positive screening test had a normal UA/C ratio and the isolated material was qualitatively normal being digested by hyaluronidase migrating as CS on electrophoresis and with GalN as principal HexN In one child receiving phenobarbitone therapy for convulsions the only abnormality was a positive screening test which was later negative when the drug had been withdrawn Qualitative examination of urine on both occasions gave similar results suggesting that the positive screening test while on drugs is due to some other factor and not abnormal GAG excretion

We are unable to comment on the abnormal results in Table 2 (Patients 31 52 and 124) since further samples have not been made available to us

GAG excretion was increased but of normal pattern except for excess glycoprotein in a patient with Marfan s syndrome and in three other bone dysplasias chondrodysplasia punctata Cornelia de Lange syndrome and Spielmeyer Vogt syndrome We are unable to comment on the results from a further eight patients with skeletal abnormalities in whom no definitive diagnosis has been made and further urine samples have not been made available to us except that GAG excretion was increased but appeared to be normal qualitatively In one infant the raised UA/C ratio was probably a reflection of the infant s age since it followed a normal developmental pattern and fell progressively into the normal range with advancing age (see Fig 1) and screening tests became negative The cause of the skeletal abnormalities is unknown and there are none of the features of the mucopolysaccharidoses

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A simplified approach avoiding time-consuming laboratory procedures such as column chromatography has been used to study glycosaminoglycan excretion in children giving a positive screening test. GAG have been isolated in crude form using cetylpyridinium chloride and analysed for hexosamine content and resistance to hyaluronidase digestion without further separation and purification. A simple electrophoretic separation has been used as supportive evidence for any abnormality detected.

Use of this simplified approach has been of value in the diagnosis of mucopolysaccharidoses. Children with false positive screening test can readily be shown to be normal excretors. A few anomalous results have been found in mentally retarded children and patients with other bone dysplasias.

More important, the methods used highlight the problem of detecting abnormal glycosaminoglycan excretion in the first year of life. Our results in this age group suggest that the full picture of abnormal excretion may be a late development in the mucopolysaccharidoses and where there is a high index of suspicion that an infant may have one of these disorders a full detailed analysis is desirable. The simple methods used here are of value in this respect since they are less time consuming than other methods and are easily undertaken by routine hospital clinical chemistry departments without specialized research facilities and staff.

ACKNOWLEDGEMENTS

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Key words: Mucopolysaccharidoses, Hurler's syndrome, Sanfilippo syndrome, Scheie's syndrome, bone dysplasias, glycosaminoglycan excretion, children, screening test

In the ten children with mucopolysaccharidoses the chemical findings were consistent with the clinical and radiological diagnosis However in the three infants in whom such a diagnosis was suspected the results were less easy to interpret One infant with suspected Hurler syndrome had a positive CPC screening test but a negative acid albumin test and normal UA/C ratio for her age (No 43 Table 3) Further analysis was certainly abnormal with a high proportion of hyaluronidase resistant material Clinical and radiological features left little doubt that this was a case of Hurler syndrome and metachromatic granules were demonstrable in peripheral blood lymphocytes We will never know the true significance of the urinary findings as this patient has since died but it seems that a full qualitative abnormality of GAG excretion may follow and not precede radiological changes in this condition

The second infant (No 89 Table 4) gave less clinical evidence of Hurler s syndrome and metachromatic inclusions were not present in peripheral blood lymphocytes or in skin fibroblast culture Hexuronic acid excretion was also normal and the high proportion of hyaluronidase resistant material could be explained by the heavy glycoprotein excretion demonstrated by electrophoresis We will be following this infant with great interest to see if the comments made in the first infant also apply

The third infant showed a positive CPC citrate and acid albumin test associated with a very high UA/C ratio A diagnosis of GM_1 gangliosidosis was entertained but excluded on finding normal leucocyte β galactosidase activity (18) The GAG excretion pattern was abnormal in having a high proportion of hyaluronidase resistant material but an incomplete picture of Hurler s syndrome Electrophoresis showed a prominent band migrating as hyaluronic acid The presence of this particular GAG rather than undersulphated HS or CS was further suggested by the proportion of GluN which was consistent with the total HA and HS found and the proportion of hyaluronidase digested material which corresponded to the amount of HA on electrophoresis

The simplified approach to GAG excretion studies used in this work has proved useful in the diagnosis of the mucopolysaccharidoses except in infancy It is possible that abnormal GAG excretion is a late development of this group of disorders and detailed qualitative examination of isolated material is indicated in infancy even when screening tests are negative if a mucopolysaccharidosis is suspected This is especially true if the acid albumin test is negative since this test is less reliable than CPC citrate turbidity The latter test gives false positive results and children with malabsorption and children on drugs such as antibiotics and anticonvulsants frequently give positive results which probably do not require further investigation unless there is evidence of mental retardation or skeletal anomalies

The incidence of a positive screening test associated with raised UA/C ratio in other children with a variety of skeletal abnormalities indicates a need to study GAG excretion in depth in other bone dysplasias and to follow positive screening results with care although a known mucopolysaccharidosis may seem an unlikely diagnosis The further methods of analysis required are within the compass of most clinical chemistry laboratories exclude time consuming column chromatography and demand less skill than methods used in research laboratories Perhaps an alternative method to gas chromatography for hexosamine analysis would be desirable since equipment for this may not always be available in smaller laboratories Using these methods only a small number of samples defy interpretation and require more sophisticated techniques for analysis

SUMMARY

Experience with a simple cetylpyridinium chloride screening test for detection of excess urinary glycosaminoglycans (GAG) is described The causes of false positive results are listed

A simplified approach avoiding time consuming laboratory procedures such as column chromatography has been used to study glycosaminoglycan excretion in children giving a positive screening test GAG have been isolated in crude form using cetylpyridinium chloride and analysed for hexosamine content and resistance to hyaluronidase digestion without further separation and purification A simple electrophoretic separation has been used as supportive evidence for any abnormality detected

Use of this simplified approach has been of value in the diagnosis of mucopolysaccharidoses Children with false positive screening test can readily be shown to be normal excretors A few anomalous results have been found in mentally retarded children and patients with other bone dysplasias

More important the methods used highlight the problem of detecting abnormal glycosaminoglycan excretion in the first year of life Our results in this age group suggest that the full picture of abnormal excretion may be a late development in the mucopolysaccharidoses and where there is a high index of suspicion that an infant may have one of these disorders a full detailed analysis is desirable The simple methods used here are of value in this respect since they are less time consuming than other methods and are easily undertaken by routine hospital clinical chemistry departments without specialized research facilities and staff

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PROPER LOCATION OF UMBILICAL VENOUS CATHETERS BY INTERNAL ELECTROCARDIOSCOPY

SANDRO NODARI ROCCO AGOSTINO and GIOVANNI BUCCI

From the Institute of Paediatrics Neonatal Division University of Rome Italy

In the newborn the location of the tip of an umbilical venous catheter in or near to the right atrium is necessary if the central venous pressure is to be measured and is desirable if the catheter is to be used for intravenous infusion therapy or exchange transfusion In fact hepatic and portal lesions have been frequently reported after umbilical vein catheterization (5 7 9 12 14) and it has been suggested that such lesions can be avoided at least in part if the catheter tip is located in the inferior vena cava or the right atrium (4 8 12)

Current methods for proper location have several disadvantages Changes in venous pressure from the portal to the intrathoracic region may be difficult to interpret (1 8) Roentgenographic assessment after positioning (2 11) involves exposure to radiation and additional manipulation and X ray studies if improper location of the catheter is found Positioning under image intensifier fluoroscopy often involves transportation of the infant to a distant room

Electrical potentials inside the cardiac chambers have been investigated previously in pathophysiological studies (6 13) and also used for accurate placement of the catheter in the right atrium during shunt operation for hydrocephalus (10) In the present paper a method will be presented which facilitates the location of the catheter tip in the inferior vena cava near the right atrium by means of internal electrocardioscopy using standard cardio monitoring equipment

MATERIAL AND METHODS

Newborn infants requiring intravenous therapy were selected for the study A polyvinylchloride radiopaque catheter (Argyle umbilical artery catheter size 5 Fr) was fitted to a metal connector and a plastic syringe and was filled with 2 M NaCl solution With the baby inside the incubator the left recording electrode was taped on the left mid axillary line at the 5th–6th intercostal space and the other recording electrode provided with a metal clamp connected to the metal connector of the catheter A ground electrode was also applied The electrodes were connected to a preamplifier an oscilloscope and a recorder in series (Fig 1) All electrically driven instrument close to or connected to the infant were grounded to a common ground Under sterile conditions the umbilical cord was cut about 2 cm from the skin and the catheter introduced and advanced in the vein (or subsequently withdrawn) step by step At convenient steps cardiac potentials were observed and recorded and the length of the introduced catheter measured When as judged from electro cardioscopic findings repeated attempts to enter the heart chambers were unsuccessful the catheter was advanced until a definite resistance was

In the present study the Hewlett Packard mod 780 7A & 7803 A monitors and 1500 A electrocardiograph were used Accidents were never observed during the procedure However it must be stressed that such instruments although safe for external monitoring do not fully meet present safety requirements for use with internal electrodes because of insufficient patient isolation Current U S A safety requirements (as specified by U L rules) include patient isolation limiting the possible current flow through th patient to not more than 10 μA Instruments meeting these requirements (or kits enabling older instruments to meet present requirements) are presently available from H & P as well as from other manufacturers

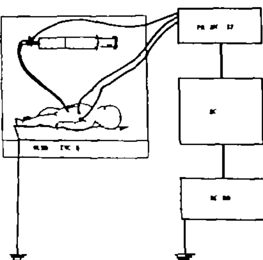


Fig 1 Diagram of the set up

felt an antero-posterior and/or lateral roentgenogram was taken and the catheter was then completely withdrawn or left inside by a length of 2–3 cm If intracardiac electric patterns were obtained the catheter was withdrawn until typical atrial patterns (see below) disappeared it was then secured its length measured and roentgenograms taken Following the results obtained in the first part of the investigation in the last part of the study the catheter was located in the position where small peaked P waves (see below) were elicited At the end of the procedure 1 ml of fluid was withdrawn in order to remove most of the hypertonic solution and the catheter flushed with 10% glucose In order to suppress a c interference it was found convenient to tape the plastic syringe onto the incubator top to maintain a completely dry field and to avoid air bubbles in the catheter and at the catheter tip It was not possible to use a 3 5 Fr catheter because of poor conductivity

RESULTS

The procedure was performed in 57 infants (birthweight range 0 6–4 4 kg postnatal age range 2–52 hours) The heart chambers were entered in 37 (63) infants (in 30 at the first attempt and in 7 after two or more attempts) This was grossly apparent by a sudden change in profile and by an increase in amplitude of electrocardiac potentials P wave patterns (Fig 2) were the most useful findings for the proper location of the catheter tip Typical atrial patterns were represented by peaked P waves with amplitude not less than 30 and usually between 50 and 100 of the QRS complex (Fig 1 A B and C) Positive atrial P waves were the most common finding (in 79 of the cases) followed by negative (66 %) and diphasic (21) atrial P waves These patterns could occur alone or together but whenever a sequence could be demonstrated on withdrawal of the catheter the positive P waves always preceded the diphasic and/or negative P waves and the diphasic P waves always preceded the negative ones When the catheter was further withdrawn small (i e with amplitude of 2–3 mm) peaked either positive diphasic or negative P waves were seen (Fig 2 E) Peaked P waves with intermediate amplitude between the atrial and the small peaked P waves were also observed in about 50% of the cases (Fig 2 D) On further withdrawal following the small peaked P wave pattern a constant pattern was observed with small and rounded or absent P waves (Fig 2 G) On deep penetration of the catheter other patterns were occasionally elicited They included small peaked P waves following atrial P waves (presumably because the tip had entered a vein after crossing the atria) atypical atrial pattern with marked elevation of the ST segment (presumably because the catheter tip was pressing against the endocardium—Fig 2) ventricular pattern In 20 infants the catheter was secured at the position in which small peaked P waves were seen and at roentgenographic examination the catheter tip was found between 0 5 cm above and 1 0 cm below the diaphragm Whenever the catheter had been located before or after the position corresponding to small peaked P waves the tip was found to be 2 or more cm below respectively 1 or more cm above the diaphragm In one additional infant only small peaked P waves followed by atypical atrial patterns could be elicited and on the film the catheter tip was found in the inferior vena cava the retrospective interpretation of this case was that the catheter tip became blocked at the entrance of the right

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ve care nurseries that it does not involve exposure to radiation and that it allows a proper location of the catheter during the catheterization procedure The latter advantage is particularly important since repeated manipulations after the initial procedure are avoided and the rate of success of atrial catheterization may be increased In view of these considerations we believe that the present procedure has definite advantages over the methods so far used for the same purpose

However it must be fully realized that by the present method a highly conductive pathway is established between the heart and instruments or other conductive material outside the body Under such circumstances electrical hazards from low amperage currents are extremely important possibly more so in very small and sick infants (3) It is therefore necessary to use instruments limiting the possible current flow through the patient to le s than 10 μA and that all electrically driven equipment close to or connected to the infant be properly grounded

SUMMARY

A method is described which facilitates the location of the tip of an umbilical venous catheter in the inferior vena cava near the right atrium by means of internal electrocardioscopy using standard umbilical catheters and cardiomonitoring equipment Entering the atria was chiefly indicated by tall and peaked P waves On withdrawal of the catheter small and peaked P waves were elicited at a radiological position of the catheter tip between 0 5 cm above and 1 0 cm below the diaphragm presumably corresponding to the inferior vena cava near the atrial inlet The latter location was obtained in 38 (67) out of 57 newborns undergoing the procedure The present method is simple and accurate and it is considered superior to methods used so far for the same purpose The need for safety precautions against electrical hazards has been stressed

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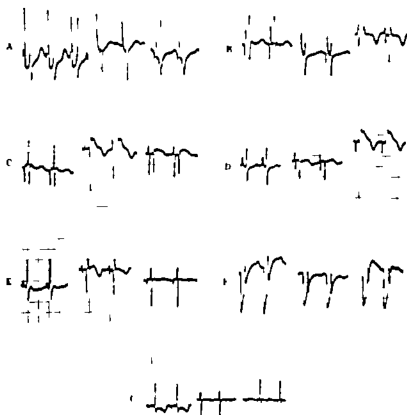


Fig 2 Most relevant tracings obtained by internal electrocardiography (A to C) typical atriograms with positive (A) diphasic (B) and negative (C) P waves (D) Intermediate peaked P wave pattern (E) Small peaked P wave pattern used for proper positioning of the catheter tip (F) Atypical atriograms (G) Patterns found on withdrawal after the small peaked P wave pattern

atrium In the remaining 19 infants in spite of repeated attempts atrial patterns were never obtained and the roentgenogram showed that the catheter had entered branches of the portal vein or other abdominal veins In 5 (26%) of these infants the catheter had been easily advanced by more than 23 (from 23 2 to 30 3%) of the total body length

DISCUSSION

According to previous studies on children and adults (6 13) intraatrial electrograms are characterized by large and peaked P waves which are negative high in the atrium diphasic in mid cavity and positive low in the chamber By the present method a completely reverse sequence was found presumably because of differences in the lead system Also at variance with the findings in older subjects the complete sequence of P wave changes was rarely elicited in our small patients and minimal movements or even rotation of the catheter often resulted in marked changes of the P wave patterns presumably because of the smallness of the atrial cavities For this reason and also because no clearcut distinction can be made between left and right intraatrial electrograms (13) we did not aim to locate the catheter tip inside the atrial cavity Luckily enough we were able to identify a P wave pattern (the small peaked P wave pattern) which could be easily recognized on the scope and which allowed the location of the catheter tip between 0 5 cm above and 1 0 cm below the diaphragm This radiological position indicates that the catheter tip is in the inferior vena cava close to the right atrium or at most in the atrial cavity very close to the venous inlet (11) a satisfactory position for venous pressure measurements and infusion therapy (8) The present method also has other advantages i e that it can be easily performed with equipment currently available in inten

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HAEMORRHAGIC CYSTITIS COMPLICATING CYCLOPHOSPHAMIDE TREATMENT IN CHILDREN

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Cyclophosphamide is a cyclic phosphoric acid ester originating primarily from nitrogen mustard gas The substance is activated in the organism forming cytostatically active compounds Besides the well known haematological side effects of cytostatic drugs cyclophosphamide has in addition been reported to cause haemorrhagic cystitis in frequencies between 3 and 40% in adult series (3 6 8 11) Corresponding figures concerning children are lacking despite the fact that during the last decade the use of the drug in paediatrics has increased

The present report deals with cyclophosphamide therapy and haemorrhagic cystitis in childhood One of the reported cases in addition stresses the fact that this kind of complication might induce serious situations if not given heed to in time

CASE REPORTS

Case 1

K L male 11 years of age when admitted to hospital due to macroscopic haematuria On admission a solid orange sized tumour was palpated above his pubic symphysis

Hb 5 6 g 100 ml Body weight 28 kg Intravenous pyelogram (IVP) revealed normal conditions Micturition cystography visualized the tumour curving into the bladder

At laparotomy the tumour was found to originate from the mesentery of the distal part of the ileum It was rooted to the sacrum and descending colon and was also adherent to the back wall of the urinary bladder The tumour was removed together with the involved part of the ileum colon and lymphatic glands Microscopic examination of the specimen revealed reticular cell sarcoma The margins of the resected intestines were free of tumour but one lymphatic gland was affected

Cytostatic treatment including cyclophosphamide and vincristin was started postoperatively A metastatic tumour was found 8 months after the first operation engaging the pelvis and left ureter and adherent to the rectum The tumour was removed

14 months after the cytostatic treatment had started the patient developed intermittent macroscopic haematuria The cyclophosphamide dose was halved Another 3 months later the boy was admitted with haematuria and discharge of blood clots. At that time the boy had had more than a total of 12 g of cyclophosphamide and 24 mg vincristin

On admission endoscopy revealed large amounts of blood clots Open evacuation and bladder drainage was performed but without improvement During this procedure careful examination demonstrated no tumour growth in the mucosa but pronounced o dema and diffuse capillary bleeding

Two days later another cystostomy had to b performed to release urinary obstruction and anuria caused by reappearance of blood clots Intravenous antifibrinolytic therapy was instituted Over the next few days th patient complained of serious pain suprapubically and in his left flank Cystography revealed free reflux bilaterally and reappearance of blood clots in the urinary bladder Cystostomy was performed again and large amounts of clotted blood were evacuated This time two Malecot catheters were inserted and treatment with continuous bladder lavage was started At the same time the antifibrinolytic treatment was concluded The bleeding gradually decreased and bladder lavage was terminated . weeks later wh n only microscopic haematuria remained

During follow up over 2 years since the last cystostomy th boy has b-- in a good condition He had no macroscopic haematuria The bladder capacity however was small due to pronounced fibrosis of the bladder wall Opaque medium radiography revealed the bladder wall measuring more than 2.5 cm No further relapse of macroscopic haematuria had occurred Haematological and urinary laboratory data and renal function tests were within normal limits

The Editorial Board has asked N R Lundstrom M D and K Lindstrom D Sc to comment on this article

The danger of electrical shock has always been present in hospitals but the risks of electrocution have increased rapidly in the past decade Ironically the risks have risen for the same reason that the quality of some types of medical care has improved the insertion into the body of catheters that transmit data on physiological parameters is becoming routine The catheters bring vital information from the body but they can also bring current into the body[1] The highest electrical risk to a patient occurs when an electrode is located inside the heart as during cardiac catheterisation Even minute leakage currents for example resulting from a defective earth connection can cause the heart to go into ventricular fibrillation

The method described by Nodari et al opens a direct road to the heart for electrical leakage currents We would therefore strongly advise against the use of old ECG equipment for these investigations and recommend every physician to practice the following precautions

1) Discuss possible hazards involved (2) with the biomedical engineer of your hospital or a representative for the equipment manufacturer The common use of ECG together with other medical instruments creates special hazards to be eliminated

2) Make use of modern earth free ECG monitors (3) such as the Hewlett Packard model 7830 A or equivalent

3) Arrange for periodic inspection of all medical electronic equipment and the power line system to prevent serious accidents Experience has shown that even new equipment must be tested (1)

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Reluctance to comply with these prescriptions may be overcome by introducing a salt rich diet or by the supply of sodium chloride tablets

Various kinds of bladder lavage have been tried prophylactically such as bicarbonate thiosulfate solutions (5) and acetyl cysteine in 20 % solution (17) The latter will neutralise the components of cyclophosphamide without having any harmful effect in itself

Cyclophosphamide treatment must be withdrawn as soon as haematuria has been verified Even after this withdrawal a high fluid intake must be maintained to diminish the risk of clot formation Heavy bleeding may be followed by bladder tamponade and require surgical intervention Blood clots can be extracted through the endoscope In children this possibility is limited due to the size of instruments Repeated cystostomies and extraction of blood clots may be necessary as in case 1 In our experience drainage through two Malecot catheters proved most efficient The catheters chosen must be large enough and be placed close to the ventral wall of the bladder We used catheters of size 18 Charriere one for drainage and one for bladder lavage Treatment by electrocoagulation (4) cutaneous ureterostomy and cystectomy (1) has also been reported In our experience antifibrinolytic treatment was of no value This concept agrees with earlier reports that bleeding from telangiectatic changes in the bladder wall is not influenced by antifibrinolytic therapy (2 14)

Early diagnosis and withdrawal of cyclophosphamide therapy may give rise to a return of symptoms as illustrated in case 2 On the other hand the metabolites of cyclophosphamide may cause irreversible fibrosis of the bladder wall This occurs when the total dose administered exceeds 6 g cyclophosphamide/m body area (9) In view of these conclusions the prognoses in our case 1 can be regarded as rather serious In case of late diagnosis multiple blood transfusions have been necessary even death caused by haemorrhages has been reported (7 12) Transient injuries to the renal tissue have also been demonstrated (13)

SUMMARY

Haemorrhagic cystitis complicating cyclophosphamide treatment is a serious complication The bladder irritation is considered to be a consequence of direct contact between bladder wall and urine containing metabolites of cyclophosphamide The early symptoms are vesical tenesmus pollakisuria and haematuria Endoscopy is the essential way of diagnosing the cyclophosphamide cystitis High daily fluid regimen and frequent voiding must be recommended as the prophylaxis of choice Moreover the drug must be administered as a single 24 hour dose in the morning Surgical techniques differ according to the severity of the bleeding Administration of an anti fibrinolytic agent proved ineffective

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Case 2

L M female 14 years of age when admitted to hospital because of albuminuria haematuria and high sedimentation rate Renal biopsy spoke in favour of a nephrotic syndrome of "minimal lesion type" Corticosteroid therapy was tried for 6 months without amelioration Following that she was given cyclophosphamide for a few days (5 mg/kg/day then 2 5 mg/kg/day) When no persistent recovery was found after 5½ months the cyclophosphamide treatment was abandoned The total cyclophosphamide amount given was 20 g corresponding to 14 g/m² body area

1 week after the cyclophosphamide treatment was finished the patient noticed macroscopic haematuria There was no dysuria The IVP revealed normal conditions in the upper urinary tracts but pronounced thickening of the bladder wall At endoscopy papillomatous changes were found in the bladder mucosa and in addition epithelial bleeding and fibrin-coated ulcers Microscopic examination of the urine revealed pathological cells

The girl was confined to bed and treated with high fluid intake and for a short time antifibrinolytic therapy Her haematuria successively decreased Endoscopy after 4 months revealed normal conditions No haematuria whatsoever occurred during a further 5 months

DISCUSSION

Through radioactive tracing of cyclophosphamide it has been found that 70% of the substance is excreted in the urine within 72 hours (18) It has been experimentally demonstrated that the irritation and changes in the bladder wall are consequences of the intimate contact between cyclophosphamide metabolites and the bladder mucosa

Accordingly when cyclophosphamide was instilled directly into the bladder no changes were seen (16) Nor did changes frequently occur in other parts of the urinary tract through which the urine only passes

The doses of cyclophosphamide recommended amount to 20–40 mg/kg every other week as shock doses or between 2 and 6 mg/kg/day in long term therapy The doses administered in the actual cases were approximately within these limits In case 1 the cyclophosphamide dose was 5–7 mg/kg/day as a long term therapy alternating with 17 5 mg/kg every other week as shock doses In case 2 cyclophosphamide was given initially 5 mg/kg/day and then 2 5 mg/kg/day

Warning signs of cyclophosphamide induced haemorrhagic cystitis are urgent and frequent voidings associated early on with microscopic haematuria The haematuria may progress in intensity to profuse and life threatening bleeding as in case 1 Urinary cultures IVP and renal function tests are usually normal (15) An early symptom in our cases was macroscopic haematuria This had been demonstrated as part of the disease before the cyclophosphamide treatment was started in both cases However the symptomatic interregnum the findings on endoscopy and the radiologically visualized swelling of the bladder wall confirm these symptoms as being significant for haemorrhagic cystitis

Haemorrhagic cyclophosphamide cystitis is confirmed mainly by endoscopy The characteristic findings are dilated vessels telangiectatic changes and submucosal haematomas Blood clots of varying age may be interpreted as tumour masses and even the microscopic differential diagnosis can be difficult (19 20) Neoplasm of the urinary bladder is extremely uncommon in children In case 1 however the tumour was localized closely to the bladder and overgrowth therefore could be expected The explorations of the bladder excluded this possibility

Cyclophosphamide haemorrhagic cystitis is a consequence of the metabolites of the agent and the method of administration Accordingly prophylactic steps through meticulous dosage and careful urinary controls are of the greatest value In our experience no limits of the total dose given can be set beyond which the risk of haemorrhagic cystitis would increase The necessity of a high fluid intake has been emphasized (10 12 15) Furthermore it has been recommended that the patient must take the drug in the morning in one single dose and that they must be requested to void frequently By these procedures the concentration of cytostatic metabolites in the urine will be low and they will be only in brief contact with the bladder mucosa Cooperation and close control over the fluid intake and voiding frequency is important in the case of children

Reluctance to comply with these prescriptions may be overcome by introducing a salt rich diet or by the supply of sodium chloride tablets

Various kinds of bladder lavage have been tried prophylactically such as bicarbonate thiosulfate solutions (5) and acetyl cysteine in 20 solution (17) The latter will neutralise the components of cyclophosphamide without having any harmful effect in itself

Cyclophosphamide treatment must be withdrawn as soon as haematuria has been verified Even after this withdrawal a high fluid intake must be maintained to diminish the risk of clot formation Heavy bleeding may be followed by bladder tamponade and require surgical intervention Blood clots can be extracted through the endoscope In children this possibility is limited due to the size of instruments Repeated cystostomies and extraction of blood clots may be necessary as in case 1 In our experience drainage through two Malecot catheters proved most efficient The catheters chosen must be large enough and be placed close to the ventral wall of the bladder We used catheters of size 18 Charriere one for drainage and one for bladder lavage Treatment by electrocoagulation (4) cutaneous ureterostomy and cystectomy (1) has also been reported In our experience antifibrinolytic treatment was *of* no value This concept agrees with earlier reports that bleeding from telangiectatic changes in the bladder wall is not influenced by antifibrinolytic therapy (2 14)

Early diagnosis and withdrawal of cyclophosphamide therapy may give rise to a return of symptoms as illustrated in case 2 On the other hand the metabolites of cyclophosphamide may cause irreversible fibrosis of the bladder wall This occurs when the *total* dose administered exceeds 6 g cyclophosphamide/m body area (9) In view of these conclusions the prognoses in our case 1 can be regarded as rather serious In case of late diagnosis multiple blood transfusions have been necessary even death caused by haemorrhages has been reported (7 12) Transient injuries to the renal tissue have also been demonstrated (13)

SUMMARY

Haemorrhagic cystitis complicating cyclophosphamide treatment is a serious complication The bladder irritation is considered to be a consequence of direct contact between bladder wall and urine containing metabolites of cyclophosphamide The early symptoms are vesical tenesmus pollakisuria and haematuria Endoscopy is the essential way of diagnosing the cyclophosphamide cystitis High daily fluid regimen and frequent voiding must be recommended as the prophylaxis of choice Moreover the drug must be administered as a single 24-hour-dose in the morning Surgical techniques differ according to the severity of the bleeding Administration of an anti fibrinolytic agent proved ineffective

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ETHANOL IN THE PREVENTION OF NEONATAL HYPERBILIRUBINAEMIA

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Haemolytic disease is the most common cause of neonatal hyperbilirubinaemia Hyperbilirubinaemia may also occur in connection with haemorrhage infections and after the administration of certain drugs It is well known that if the serum indirect bilirubin level exceeds 20 mg/100 ml manifest kernicterus may develop On the other hand the risks of exchange transfusion have stimulated the search for alternative methods of reducing serum bilirubin levels to prevent brain damage Phenobarbital and phototherapy have received the greatest attention Both have been found to lower the bilirubin values significantly compared with the control children (1 3 4 6 9 12 14)

In recent years ethanol has been used in obstetrics as an agent to prevent the premature birth (2 5) In 1969 Waltman et al reported that ethanol given to the mother prior to delivery can lower considerably the bilirubin level of the newborn (11) No new studies have been published on the use of ethanol in the prevention of neonatal hyperbilirubinaemia We decided therefore to investigate the influence of ethanol given to the mother in various quantities on the neonatal bilirubin levels

MATERIAL AND METHODS

The material was collected from the Departments of Obstetrics and Gynecology and Paediatrics, University of Oulu It consisted of 93 pregnant women whose delivery was to be induced The reason for induction was suspected postmaturity (more than 10 days) (29 gravidas) mild pre-eclampsia (49 gravidas) and essential hypertension (5 gravidas) The series also included 10 normal gravidas near term Mothers with additional disease such as diabetes Rh incompatibility twin pregnancy or severe pre eclampsia were excluded from the study and also mothers who were not delivered on the first day after induction

The mothers received the ethanol by intravenous infusion or orally the day before the induction. The interval between discontinuing the ethanol and delivery varied from 11 to 32 hours, average 25 hours The induction of delivery was performed with oxytocine infusion (5 IU/500 ml of 5% glucose) or by rupturing the foetal membranes.

The mothers were divided into five groups according to the quantity of ethanol administered

Group A 19 controls received no ethanol

Group B 35 mothers received 4 50 ml of ethanol (cognac) at intervals of 4–6 hours The total dosage was 80 g of ethanol

Group C 16 mothers received 63 g (80 ml 99 v/v %) of ethanol in 1 000 ml of physiological saline intravenously at the rate of 2 ml min

Group D 12 mothers received 94 g (1 0 ml 99 v/v %) of ethanol intravenously as in Group C

Group E 11 mothers received 102 g (130 ml 99 v/v %) of ethanol as in Groups C and D

The ethanol groups did not differ from the controls as regards the medical treatment of the mothers at the end of the pregnancy No barbiturates were used

The gestational ages ranged from 37 to 43 weeks though all the infants were full term including eight newborns with birth weights of less than 2 500 g The mean birth weight of the newborn was 3 6 0 g (range 030–4 950) (Table 1) The clinical examination of the neonates was performed immediately after birth and their clinical status was reassessed on the first day of life and at discharge All of the neonates were breast fed They were followed up for six days

Micro blood samples were taken from the newborns daily usually in the morning The total bilirubin was determined according to a modified Malloy Evelyn micromethod

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Group C 16 mothers received 63 g (80 ml 99 v/v %) of ethanol in 1 000 ml of physiological saline intravenously at the rate of 2 ml min

Group D 12 mothers received 94 g (120 ml 99 v/v) of ethanol intravenously as in Group C

Group E 11 mothers received 102 g (130 ml 99 /v %) of ethanol as in Groups C and D

The ethanol groups did not differ from the controls as regards the medical treatment of the moth rs at the end of the pregnancy No barbiturates were used

Th gestational ages ranged from 37 to 43 weeks though all th infants were full term including eight newborns with birth weights of less than 2 500 g The mean birth weight of the newborn was 3 620 g (range 2 030–4 950) (Table 1) The clinical examination of the neonates was performed immediately after birth and th ir clinical status was re assessed on the first day of life and at discharge All of th neonates were breast fed They were followed up for six days

Micro blood samples were taken from the newborns daily usually in the morning The total bilirubin was determined according to a modified Malloy Evelyn micromethod

Table 1 *The distribution of newborns in various groups to the gestational age and birth weight*

Group	Mean gestational age in weeks (range)	Mean birth weight g (range)	No of infants <2 500 g
Group A *N*-19	40 (37–42)	4 380 (2 350–4 950)	2
Group B *N*-35	39 (37–41)	3 560 (2 400–4 580)	3
Group C *N*-16	40 (37–41)	3 720 (2 480–4 450)	1
Group D *N*-12	39 (38–42)	3 680 (2 030–4 650)	1
Group E *N*-11	39 (38–41)	3 660 (2 450–4 350)	1

RESULTS

The general condition of the mothers was unaffected by the alcohol treatment The various groups of neonates did not differ from controls in birth weight gestational age and condition after birth (Table I)

The mean bilirubin levels of the different groups of neonates are presented in Table 2 The results were similar in all the groups The highest bilirubin level was reached on the 3rd–5th day in all the groups after which the level started to fall The mean daily bilirubin values of the groups did not show significant differences from the controls except in Group B The average bilirubin level of Group B was almost significantly ($p<0\ 05$) higher on the 6th day of life than in the control group

Hyperbilirubinaemia of more than 15 mg/100 ml was recorded in 1/11 controls 4/35 in Group B 2/16 in Group C and none in Groups D and E Exchange transfusion was performed on one newborn of Group B because of hyperbilirubinaemia and another one in the control group because of ABO incompatibility

DISCUSSION

According to this study ethanol given to the mother the day before delivery could not be proved to lower the total bilirubin level of the newborn in the first six days of life These

Table 2 *Bilirubin levels (mg/100 ml) of the newborn infants whose mothers received various quantities of ethanol the day before delivery*

Group	Day after delivery 1	2	3	4	5	6
Group A controls no ethanol *N*-19 Mean (±S D)	5 0 (±1 8)	6 8 (±3 1)	7 7 (±3 5)	7 1 (±3 7)	6 7 (±3 6)	5 1 (±2 9)
Group B 80 g ethanol per os *N*-35 Mean (±S D)	4 9 (±1 7)	7 6 (±2 5)	8 2 (±3 1)	9 1 (±4 3)	8 8 (±4 1)	7 7 (±3 1)
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Group E 102 g ethanol i v *N*-11 Mean (±S D)	4 3 (±1 2)	6 6 (±2 0)	6 8 (±3 3)	7 5 (±3 6)	7 9 (±4 2)	7 3 (±3 0)

results differ from those reported by Waltman et al (11) They had a statistically significant reduction in the serum bilirubin values on the 3rd 4th and 5th days of life of infants whose mothers were treated with 100–115 g of ethanol 3–96 hours prior to delivery In this study the bilirubin values of the newborn were not significantly lower than the control values not even in the group whose mothers received the greatest quantity of alcohol 102 g On the contrary the bilirubin values in most of the groups treated with ethanol were higher than the control values

Our series consisted principally of pregnant women with mild pre eclampsia and suspected postmaturity The patients with mild pre eclampsia were distributed relatively among all the groups including the control group It has been reported that high bilirubin values are less frequent in the newborn of toxaemic mothers than in normal newborn infants (10) However in our earlier study we found no difference between the mean bilirubin levels of the newborns of toxaemic and normal mothers (4)

The reducing effect of phenobarbital on bilirubin has been considered to be due mainly to induction caused by the microsomal enzymatic systems of the liver including glucuronidation It has been suggested that phenobarbital can also increase the bile secretion capacity and by this route increase the excretion of bilirubin from the organism as well as improve the hepatic uptake of bilirubin in other words increase bilirubin clearance (7 13) Our study suggests that a short administration of ethanol to mothers does not influence the clearance of bilirubin in the liver of newborn infants Some of the latest reports suggest that short term administration of alcohol has an inhibitory effect upon several enzymes of the liver (8) The alcohol quantities needed for inhibition corresponded to the plasma levels for moderate use of alcohol in man On the other hand regular use has been found to make the endoplasmic reticulum of human liver cells hypertrophic after 9–16 days and induce the metabolism of various drugs in the liver (8)

Although we were unable in this study to show that short term administration of ethanol has any influence on the bilirubin values of the newborn it would be interesting to discover if the newborns whose mothers had received long term alcohol treatment for premature contractions in late pregnancy have lower bilirubin levels than controls

SUMMARY

Seventy four pregnant women were given 80 g of ethanol orally or 63 94 or 102 g intravenously the day prior to delivery Nineteen mothers and their offspring served as controls The effect on the bilirubin values of the newborn was studied The bilirubin levels of the newborn were not significantly lower during the first six days of life compared with the controls The results differ from those reported earlier The reasons for this are discussed

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URINE ELIMINATION OF AN ORAL SALT AND FLUID LOAD IN HEALTHY CHILDREN

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During the past decades several reports have pointed to a causal relationship between positive sodium balance and hypertension (3 4 5 10 12) This has evoked a need for a simple test of the control of sodium homeostasis in patients both with renal and circulatory disturbances In a study in children with recurrent urinary tract infections in this laboratory (1) we have introduced a simple test of the renal handling of sodium based on the determination of hourly urinary sodium excretion of an oral salt load The test was used parallel with standard clearance studies with superimposed intravenous saline load A good correlation was found between the information on tubular sodium reabsorption given by the two tests In the present study the test of urinary sodium excretion following an oral salt load has been standardized in a series of healthy children without any signs of renal disease

MATERIAL

Thirteen children 8 boys and 5 girls were studied The age ranged between 8 and 14 years All children were hospitalized for traumatic fractures None of the children had any history or signs of renal disease or other disorders affecting water or electrolyte metabolism All children were considered normally developed Studies were performed at least 5 days after the fracture had been corrected All children were in good physical condition at the time of the study Informed parental consent was obtained in every case

METHODS

All studies were carried out after the children had been hospitalized for at least 5 days The daily sodium intake before the study averaged 100 mEq/1.73 m² BSA/day with small fluctuations All urine samples were obtained by spontaneous voiding For blood sampling and injection purposes infusion cannulas (Stille Werner AB) were inserted into two superficial brachial veins

All studies were carried out under relatively constant degrees of fluid expansion Table 1 demonstrates a protocol from a typical study For this purpose the children ingested water in an amount corresponding to 2–2.5% of the body weight during one hour and thereafter in amounts corresponding to 0.5% of the body weight every 30 min The first urine collection period was generally obtained 2–3 hours after the high fluid intake was started Urine was then collected at regular hourly intervals During the second urine collection period the oral salt load was given as tablets of sodium chloride (ACO) in the dosage of 80 mEq sodium/1.73 m² BSA in 7 children and in a dosage of 160 mEq sodium/1.73 m² BSA in 6 children The tablets were ingested during the first 15 min of the second urine collection period Two of the patients receiving the dose of 160 mEq sodium/1.73 m² BSA vomited shortly after the intake of tablets The results from those two studies are not included in this report

During water diuresis but before sodium chloride was administered a single injection of a 9% inulin (Laevosan Gesellschaft) and an 18% paraaminohippuric acid (PAH) (MSD) solution was given intravenously in the amount of 0.75 ml/kg b.w. Blood samples were withdrawn every 5 min during the first 20 min following the injection and thereafter every 10 min during another 60 min From the plasma disappearance rates so obtained the clearances of inulin (C_{In}) and PAH (C_{PAH}) could be calculated using the formula given by Sapirstein (13) Filtration fraction was calculated as the quotient between glo-

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URINE ELIMINATION OF AN ORAL SALT AND FLUID LOAD IN HEALTHY CHILDREN

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During the past decades several reports have pointed to a causal relationship between positive sodium balance and hypertension (3 4 5 10 12) This has evoked a need for a simple test of the control of sodium homeostasis in patients both with renal and circulatory disturbances In a study in children with recurrent urinary tract infections in this laboratory (1) we have introduced a simple test of the renal handling of sodium based on the determination of hourly urinary sodium excretion of an oral salt load The test was used parallel with standard clearance studies with superimposed intravenous saline load A good correlation was found between the information on tubular sodium reabsorption given by the two tests In the present study the test of urinary sodium excretion following an oral salt load has been standardized in a series of healthy children without any signs of renal disease

MATERIAL

Thirteen children 8 boys and 5 girls were studied The age ranged between 8 and 14 years All children were hospitalized for traumatic fractures None of the children had any history or signs of renal disease or other disorders affecting water or electrolyte metabolism All children were considered normally developed Studies were performed at least 5 days after the fracture had been corrected All children were in good physical condition at the time of the study Informed parental consent was obtained in every case

METHODS

All studies were carried out after the children had been hospitalized for at least 5 days The daily sodium intake before the study averaged 100 mEq 1 73 m BSA/day with small fluctuations All urine samples were obtained by spontaneous voiding For blood sampling and injection purposes infusion cannulas (Stille Werner AB) were inserted into two superficial brachial veins

All studies were carried out under relatively constant degrees of fluid expansion Table 1 demonstrates a protocol from a typical study For this purpose the children ingested water in an amount corresponding to 2 25% of the body weight during one hour and thereafter in amounts corresponding to 0 5 of the body weight every 30 min The first urine collection period was generally obtained 2–3 hours after the high fluid intake was started Urine was then collected at regular hourly intervals During the second urine collection period the oral salt load was given as tablets of sodium chloride (ACO) in the dosage of 80 mEq sodium/1 73 m BSA in 7 children and in a dosage of 160 mEq sodium/1 73 m BSA in 6 children The tablets were ingested during the first 15 min of the second urine collection period Two of the patients receiving the dose of 160 mEq sodium/1 73 m BSA vomited shortly after the intake of tablets The results from those two studies are not included in this report

During water diuresis but before sodium chloride was administered a single injection of a 9 inulin (Laevosan Gesellschaft) and an 18 para-aminohippuric acid (PAH) (MSD) solution was given intravenously in the amount of 0 75 ml/kg b w Blood samples were withdrawn every 5 min during the first 20 min following the injection and thereafter every 10 min during another 60 min From the plasma disappearance rates so obtained the clearances of inulin (C) and PAH (C) could be calculated using the formula given by Sapirstein (13) Filtration fraction was calculated as the quotient between glo-

Table 1 *Protocol from a typical oral sodium load test*

Time	Water intake	Sodium intake	Urine sample	
0	2 of body weight			
60	0 5 of body weight		Discarded	
90	0 5 of body weight		Discarded	GFR determined by single injection technique
120	0 5 of body weight			
150	0 5 of body weight	80 mEq/1 73 m² BSA	Sample 1	
180	0 5 of body weight			
210	0 5 of body weight		Sample 2	
240	0 5 of body weight			
270	0 5 of body weight		Sample 3	
300	0 5 of body weight			
330	0 5 of body weight		Sample 4	
360	0 5 of body weight			
390	0 5 of body weight		Sample 5	
420	0 5 of body weight			
450	0 5 of body weight		Sample 6	
480	0 5 of body weight			
510	0 5 of body weight		Sample 7	

merular filtration rate (clearance of inulin) and the clearance of PAH

Analytical methods

The concentration of sodium in urine was determined with a flame photometer The concentration of inulin in blood was determined by the Antrone method (8) and the concentration of PAH according to the method of Smith (14)

RESULTS

Children receiving 80 mEq sodium/1 73 m BSA

Fig 1 demonstrates the hourly urinary sodium excretion in a typical study and in Fig 2 the mean urinary sodium excretion ± one standard deviation is given from all children in the study Following the sodium load urinary sodium excretion increases significantly for 2 hours Thereafter urinary sodium excretion reaches a fairly stable level during the next 4 hours of collection No significant fall in urinary sodium excretion was seen between these 4 periods No peak excretion was observed

Table 2 summarizes the renal functional data following the oral sodium load of 80 mEq/1 73 m BSA The hourly urinary sodium

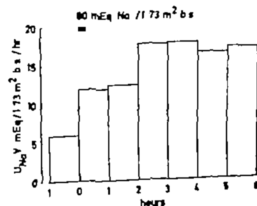


Fig 1 Hourly urinary sodium excretion following the oral sodium load of 80 mEq/1 73 m BSA in a typical study Time 0 indicates the start of sodium administration

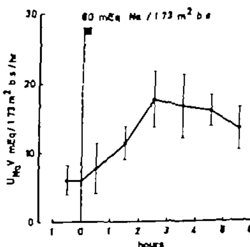


Fig 2 Mean hourly urinary sodium excretion ± one standard deviation in all children receiving the oral sodium load of 80 mEq/1 73 m BSA Time 0 indicates the start of sodium administration

Table 2 *Glomerular filtration rate (GFR) clearance of PAH (C_{PAH}) filtration fraction and urinary sodium excretion (U_{Na} V) following an oral sodium load of 80 mEq/1 73 m² BSA*

Mean values ± one standard deviation are given

GFR, ml/min/1 73 m² BSA	122 5±16 4
C_{PAH} ml/min/1 73 m² BSA	546 0±47 2
Filtration fraction,	21 2± 1 3
U_{Na} V mEq/hr/1 73 m² BSA	16 0± 1 8
6 hours accumulated U_{Na} V in % of the given load	58 7±15 9

excretion from the 3rd to the 6th hour following the administration of sodium chloride i e when the urinary sodium excretion reached a plateau averaged 16 mEq/1 73 m² BSA/hour Following 6 hours after administration of sodium chloride 58 7 % of the given load had been excreted The excreted amount has been calculated as total urinary sodium excretion 0 to 6 hours after load − hourly urinary sodium excretion before load × 6 The glomerular filtration rate (GFR) averaged 123 ml/min/1 73 m² BSA The clearance of PAH averaged 546 ml/min/1 73 m² BSA and the filtration fraction averaged 21 %

Children receiving 160 mEq sodium/1 73 m² BSA

In Fig 3 the mean hourly urinary sodium excretion in 4 children receiving 160 mEq sodium/1 73 m² BSA has been compared to the values obtained in children receiving 80 mEq sodium/1 73 m² BSA The urinary sodium excretion is higher during the whole course of the study following the higher dose of sodium

Table 3 summarizes renal functional data following the oral load of 160 mEq sodium/1 73 m² BSA. The urinary sodium excretion was increased with a factor of 1 6 in comparison with the excretion following the single dose of sodium The total increase in urinary sodium excretion during the 6 hours calculated in percentage of the given load was 49 5 The average GFR C_{PAH} and filtration fraction did not differ significantly from the results of patients receiving the single dose of sodium

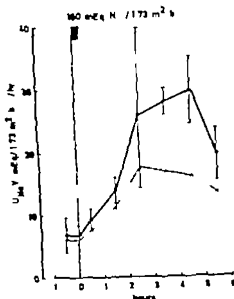


Fig 3 Mean hourly urinary sodium excretion ± one standard deviation in the children receiving the oral sodium load of 160 mEq/1 73 m² BSA As a dashed line the mean urinary sodium excretion in the children receiving the dose of 80 mEq sodium/1 73 m² BSA is given Time 0 indicates the start of sodium administration

COMMENTS

When renal tubular transport capacity of a specific substance is to be studied the kidney is generally challenged with an intravenous or an oral load of that substance Tests including oral loads and spontaneously voided urine samples are easier to perform but are thought

Table 3 *Glomerular filtration rate (GFR) clearance of PAH (C_{PAH}) filtration fraction and urinary sodium excretion (U_{Na} V) following an oral sodium load of 160 mEq/1 73 m² BSA*

Mean values ± one standard deviation are given The *p*-values stand for a comparison with the renal functional data achieved after the oral load of 80 mEq sodium/1 73 m² BSA

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Filtration fraction	22 0± 1 3	>0 5
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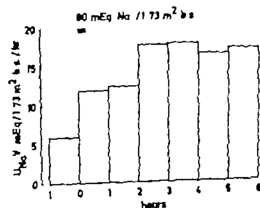


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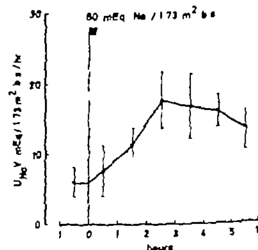


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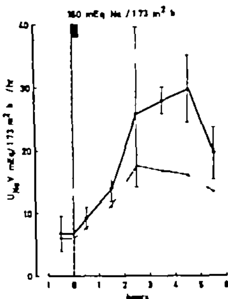


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to present greater errors of method due to variations in intestinal absorption and incomplete urine sampling Available data do however suggest that a moderate extra load of sodium exceeding the normal intake is more or less completely resorbed by the small intestine (11) The fact that no great variation in urinary sodium excretion was found among the children indicates a constant intestinal absorption When the oral load was doubled from 80 to 160 mEq sodium/1 73 m BSA the urinary sodium excretion was increased not with a factor of 2 but 1 6 The fact that the urinary sodium excretion did not completely parallel the increase in oral load can be explained by renal as well as gastrointestinal factors The higher sodium intake might have provoked a higher degree of extracellular volume expansion which is known to be assumed with a flux of sodium from the serosal to the mucosal surface of the small intestine (7 9) The urinary sodium excretion during the last 4 hours was found to be rather constant Thus the spontaneously voided urine samples performed during high fluid intake in the present study appear to give an accurate estimation of the diuresis

The method presented has been designed to evaluate the role of the kidney in the control of sodium homeostasis namely the ability to rapidly adjust to an acute positive sodium and water load As the response to the salt load in these healthy children demonstrated a consistent and characteristic pattern we found it adequate to interrupt the test after 6 hours even if only 58 • of the given dose had been excreted after that time The kidney was thus provoked with an oral fluid and salt intake that should result in a normonatremic volume expansion as well as a gain in total body sodium Both factors might have signalled the natriuretic response Since the pathways by which extracellular volume expansion and changes in sodium balance will affect urinary sodium excretion are not completely clear (6 15 16) the signal mechanisms used in the present study are impossible to predict Previous clearance studies have shown that the filtered load of sodium will not change when equivalent loads of fluid and salt are given intravenously (1) Th natriuretic response in this test must therefore be attributed to an inhibition of the tubular sodium reabsorption The study where clearance technique was used parallel to the oral salt load method were performed in children with wide variation in glomerular filtration rates (1) The reduction in glomerular filtration rate in those children was secondary to recurrent urinary tract infections In this study it was found that when glomerular filtration rate was reduced the immediate response to the oral salt load was less pronounced In fact there was a significant direct relationship between glomerular filtration rate and sodium elimination rate during an early phase i e 3–6 hours following the load The reduced ability to rapidly eliminate the salt load was however not due to the low glomerular filtration rate *per se* but rather to an inability of the renal tubules to adapt to the salt load by inhibiting the sodium reabsorption Thus in case of renal disease with reduced filtration rates there appears to be a less sensitive control of the tubular sodium reabsorption The control of tubular sodium reabsorption might however also be disturbed in the absence of reduction of glomerular filtration rate Studies in patients with aortic coarctation have also demonstrated this (2) Therefore it is suggested that the glomerular filtration rate is always determined at the time of the salt load in order to find out whether the disturbance of tubular sodium reabsorption is due to generalized renal disease with a fairly homogenous reduction of renal function or to specific renal tubular factors

SUMMARY

A method for studying renal response to an oral sodium and fluid load has been tested in a group of normal children aged 8 to 14 years An oral sodium load of 80 mEq/1 73 m BSA was given after water diuresis was induced Urine was collected hourly by spontaneous

loading At the time of the study the glomerular filtration rate and clearance of PAH was determined by the single injection technique After the salt load was given the urinary sodium excretion was increased for 2 hours Thereafter it reached a fairly stable level which was maintained at least during the next 4 hours This response was a concomitant finding in all the children studied It was thereafter considered justified to estimate the renal response to the salt load as the mean urinary sodium excretion from the third to the sixth hour after sodium administration The urinary sodium excretion was then found to be 16 ± 1 8 mEq/hr/1 73 m BSA When the salt load was doubled the urinary sodium excretion was increased by a factor of 1 6 Various explanations for this deviation from complete dose response are discussed It is suggested that the test is used in the evaluation in the control of sodium homeostasis in patients with renal and circulatory disturbances

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DISPLACEMENT OF ALBUMIN BOUND BILIRUBIN BY INJECTABLE DIAZEPAM PREPARATIONS IN VITRO

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Sodium benzoate has long been known to cause effective displacement of bilirubin from albumin and this substance has therefore been widely used as accelerator in the determination of serum bilirubin by diazo coupling (5) Recently Schiff and co workers (8) called the attention to the fact that Valium® in its injectable form is a potent displacer of bilirubin from albumin in vitro This displacing effect was found to be due to sodium benzoate which is a constituent of the buffer preservative The therapeutically active compound of the injectable preparation diazepam was not involved in the competition with bilirubin for the binding sites on the albumin molecule The authors conclude that their findings raise the possibility of kernicterus when the fixed drug combination is used in neonates

Three injectable diazepam preparations are available in Norway namely Valium® Stesolid® and Vival® Both Valium® and Stesolid® for injection contain benzoate in the buffer preservative while Vival® does not We have studied the effect of these three preparations on the bilirubin albumin binding in vitro

MATERIALS AND METHODS

The method used for testing the displacing effect on bilirubin by the drugs has previously been reported by Bratlid (1) and will not be described in detail here The principles of the method are that erythrocytes are capable of binding bilirubin (4 7) and that this binding reflects the degree of albumin binding of bilirubin (1–4)

The drugs were added to a mixture of human erythrocytes bilirubin and albumin in isotonic phosphate buffers pH 6 8 and 7 4 After incubation for 15 minutes at 37 C the amount of bilirubin bound by the erythrocytes was determined as described earlier (1)

Bilirubin (Sigma) and lyophilized human serum albumin (Kabi) were used without further purification Erythrocytes were obtained from healthy donors of various blood groups

Valium® Stesolid® and Vival® were the commercial preparations supplied by Hoffmann–La Roche Dumex and A L respectively The buffer preservatives of Stesolid® and Vival® were obtained from the manufacturers

RESULTS

As can be seen from Fig 1 both Valium® and Stesolid® increased the cellular binding of bilirubin markedly At pH 7 4 a concentration of diazepam of 90 µg/ml increased the cellular binding of bilirubin almost three times blank values By reducing the pH to 6 8 this displacing effect was greatly potentiated The same concentration of diazepam thus gave a six fold increase in cellular bound bilirubin (Fig 1) Experiments with buffer preservative of Stesolid® gave similar results as the complete preparation

Vival® slightly increased the cellular binding of bilirubin at pH 6 8 (Fig 1) This effect was also found to be due to the buffer preservative At pH 7 4 Vival® did not have any displacing effect on bilirubin

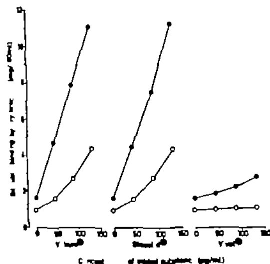


Fig 1 The effect on the binding of bilirubin by erythrocytes of the addition of increasing amounts of different injectable diazepam preparations (expressed as the concentration of diazepam in the incubate) to the incubation mixtures. Mean values of 4 experiments at pH 7 4 (O) and pH 6 8 (●) are given. The bilirubin concentration was kept constant at 9 7 mg/100 ml with a bilirubin/albumin molar ratio of 0 7

DISCUSSION

The clinical significance of having a knowledge of substances which might cause bilirubin displacement was clearly demonstrated by the observation made by Silverman et al (9) that administration of sulfisoxazole to premature infants increased the incidence of bilirubin toxicity Since that time many substances have been shown to be bilirubin displacers both *in vitro* and *in vivo* (3 6)

The findings presented in the present report are in agreement with the observations made by Schiff and associates (8) that the injectable preparation of Valium® is an effective displacer of bilirubin in vitro Furthermore we found that this is the case with Stesolid® too which also contains benzoate in the buffer preservatives On the other hand Vival® which does not contain benzoate did not show any significant displacing effect at pH 7 4 but had a slight effect at pH 6 8 This effect was also found to be due to constituents of the buffer preservatives

We do not know the concentrations of the displacing agents in in vivo situations compared to the present in vitro conditions but the displacing effect in vitro seems to be linear and at a low pH it is considerable even at low concentrations of the added preparation (Fig 1) The fact that benzoate is known to be a potent displacer of bilirubin from albumin (5) should therefore raise the possibility of untoward effects of Valium® and Stesolid® in the neonate with hyperbilirubinemia and a tendency to acidosis

Diazepam is so valuable in the treatment of convulsions and as a relaxant for infants artificially ventilated that it cannot be omitted from the therapeutic armament In neonatal medicine however preparations which do not contain benzoate should be preferred for parenteral administration as long as exact knowledge of the serum levels of benzoate after parenteral administration is lacking

SUMMARY

Three injectable diazepam preparations Valium® Stesolid® and Vival® were tested for their ability to displace bilirubin from albumin in vitro Both Valium® and Stesolid® caused marked displacement of bilirubin and this effect was potentiated by a reduction in pH Both these diazepam preparations contain benzoate in the buffer preservative Vival® which does not contain benzoate in the buffer preservative did not show any displacement at pH 7 4 and had only a slight effect at pH 6 8

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Key words *Bilirubin bilirubin displacement, diazepam kernicterus neonates*

ADDENDUM

Since these results were submitted for publication in December 1972 Dumex has changed the buffer preservative of the injectable diazepam preparation Stesolid[R] The new preparation which does not contain benzoate will be marketed soon With the same test system as used in the present report this new preparation has been found not to give displacement of bilirubin from albumin

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LINEAR GROWTH RATE BONE MATURATION AND GROWTH HORMONE SECRETION IN PREPUBERTAL CHILDREN WITH CONGENITAL ADRENAL HYPERPLASIA

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Since Wilkins contribution (21) cortisone or hydrocortisone has been the treatment of choice in congenital adrenal hyperplasia In spite of good results in individual cases a general trend was reported toward growth retardation b fore puberty with heights below normal mean for age (1 3 16) In a previous publication we focused attention on infancy period when overtreatment appeared to be frequent (10) If one achieves correct adrenal suppression according to urinary excretion of 17 keto steroids and (or) pregnanetriol it is often difficult to avoid pharmacological effects of hydrocortisone on growth This study was designed to evalute the effect of various hydrocortisone dosages on linear growth and bone maturation in a group of congenital adrenal hyperplasia affected prepubertal children Such study depends principally on methodological difficulties which are discussed and should be taken into account for the therapeutic evaluation

MATERIALS AND METHODS

Twenty one children (14 girls and boys) with congenital adrenal hyperplasia due to 1 hydroxylation defect were studied Fifteen were salt losers diagnosed early No reference will be made to mineralocorticoid treatment All received oral hydrocortisone with two-thirds of the daily dosage given in the evening Dosage was determined in each case as the minimum capable of maintaining urinary pregnanetriol in a normal range for age according to Jayle et al (4) Periods with repeated high urinary pregnanetriol excretion were excepted In late diagnosed cases with advanced bone maturation slight overtreatment was decided according to a schedule described further in this paper Detailed conditions of clinical management were previously described (10)

The growth studies were performed when the patients were between the ages of 3 and 10 years A minimal follow up duration of 2 years was required Patients were seen every 6 months *Age limits* for this study were chosen in order to eliminate the infancy period and the pubertal growth spurt Female standards for growth velocity were used for the whole group including 6 boys since in that period of life differences are minimal Longitudinal data (20) have shown that over the age of 3 to 10 years normal children very seldom have a large change of growth rate The same was shown for bone maturation rate although suitable velocity standards are not available (18)

The height was measured according to the method of Sempe & Masse (14) with reference to the standards of the Centre d'Etudes de la Croissance et du Developpement de l'Enfant Centre International de l'Enfance For each child a smooth curve was drawn through the plotted measurements Deviation from normal was presented in terms of Standard Deviation Scores (SDS) (19) Annual height increments were read-off at 1 year intervals and considered adequate even though the growth response on treatment was not really linear The mean hydrocortisone dosage was calculated for each year and expressed in milligrams per square meter of body surface per day

The skeletal maturity was estimated from the left

Medical School of Endocrinology University of Barcelona Spain on fellowship of the Ministère des Affaires Etrangères (France)

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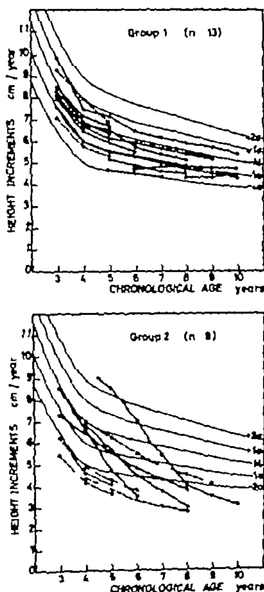


Fig. 1 Annual height increment curves in children treated with oral hydrocortisone normal growth rates (group 1) and reduced growth rates (group 2) plotted against chronological ages for twenty two periods of treatment Periods with full lines belong to the study of bone age and height increments (n 15)

hand and wrist X rays using the numerical method of Tanner et al (18) modified by Sempe (13) It was correlated with chronological age (CA) and height A careful follow up of 2 years or more with X rays taken *every* 6 months allowed precise evaluations numerical values were plotted against CA on French standards for bone maturation and at 1 year intervals skeletal maturation was read off and expressed as bone age Bone age increments were calculated for each year and referred in text as *bone age per year*

The effect of hydrocortisone on growth was estimated on mixed longitudinal data and on individual distance curves

1 Mixed longitudinal data (a) For 19 children individual curves of annual height increments were distributed into two groups according to the growth increment SDS difference measured between onset and end of each period of treatment (19) For 3 children out of these 19 the curves were divided into two periods according to growth rate so that a total of 22 periods ($n = 22$) were studied In group 1 ($n = 13$) the mean SDS difference was 0 ± 0.2 in group 2 ($n = 9$) its value was 2 ± 0.6 (M±S E) corresponding to decreasing growth rates A careful follow up of skeletal maturation was possible for 13 children out of 19 (8 in group 1 3 in group 2 and 2 having part of growth curves in both groups) Periods of treatment corresponding to this study are presented on Fig 1 For each year of treatment linear growth bone maturation and mean hydrocortisone dosage were evaluated

(b) For the same 19 children the slope of the linear growth curve was calculated when chronological age and height were represented in a logarithmic scale Values of the slopes were then correlated with mean values of hydrocortisone dosages delivered during the whole period on semi logarithmic representation in order to express a dose–response relationship

2 Individual distance curves In order to assess the effect of treatment on height between ages of 3 and 10 years distance curves were used (a) Ten patients had been treated since the first trimester of life Before the age of 18 months hydrocortisone dosages had been lower than 50 mg/m per 24 hours in 4 cases higher in 6 cases After the age of 18 months all patients received dosages lower than or equal to 30 mg m per 24 hours Height deviation from normal was expressed by standard deviation scoring (19) and the growth status achieved at the time of the present study was evaluated (b) Three patients were first treated after infancy Higher dosages of hydrocortisone were used in order to block skeletal maturation The effect on height and bone age was shown on individual curves plotting the height against chronological age and against bone age Treatment was monitored in order to reduce the difference between chronological age and bone age without producing symptoms of corticoid overtreatment as excessive weight gain moon like faces or hypertension

The plasma immunoreactive growth hormone was measured after stimulation by a sequential arginine insulin test (8) using the modified method of Rosselin (11) with adsorbant precipitation (12) The lower limit for normal peak values in our laboratory is 5 ng ml

RESULTS

Effect of hydrocortisone on annual height and bone age increments

The annual height increment curves corresponding to twenty two periods of treatment were plotted on Fig 1 They were divided into two groups group 1 ($n = 13$) with normal and group 2 ($n = 9$) with reduced growth rate The

annual bone age and height increments were calculated for fifteen periods of treatment (10 in group 1 and 5 in group 2) among 13 children (Fig 2) In group 1 corresponding to a total of 35 treatm nt years mean hydrocortisone dosage was 25 1 ± 0 9 mg/m per 24 hours M ± S E 15–36 mg/m per 24 hours) Most dosages were equal to or below 30 mg/m per 24 hours In group 2 with a total of 15 treatment years mean hydrocortisone dosage was 36 5 ± 2 1 mg/m per 24 hours (range 27–55 mg/m per 24 hours) Bone age increments per year showed variations parallel to changes in growth rate The mean annual bone age increment was 0 82 ± 0 07 years in group 1 and 0 47 ± 0 06 in group 2

Effect of hydrocortisone on linear growth rate and dose response relationship

For each period of treatment ($n = 22$) the value of the slope of the linear growth curve was calculated 0 47 ± 0 01 (M ± S E range 0 40–0 50) in group 1 and 0 29 ± 0 02 (0 22–0 40) in group 2 There was a significant negative cor

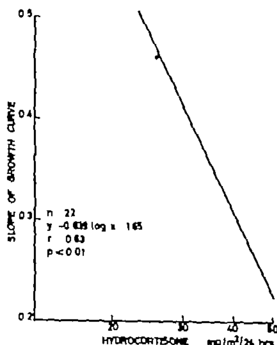


Fig 3 Correlation between mean hydrocortisone dosages and slopes of linear growth curves for twenty two periods of treatment

relation (r −0 63 $p < 0.01$) with mean hydro cortisone dosages (Fig 3) A diminished l near growth rate expressed as a lower slope value for the growth curve was observed in nine periods of treatment out of ten when hydro cortisone dosage was between 30 and 45 mg/m per 24 hours

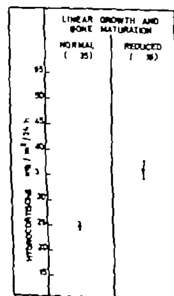


Fig 2 Oral hydrocortisone dosages related to linear growth and bone maturation rates evaluated for each year in fifteen periods of treatment normal rates (group 1) and reduced rates (group 2) (n = number of years of treatment) Mean ± S E

Linear growth and bone maturation of children treated from early infancy

Ten patients were followed up for periods of 2 to 8 years (Fig 4) All received hydrocorti sone dosages inferior to or equal to 30 mg/m per 24 hours after the age of 18 months Heights recorded between birth and age of 1 month were between 48 and 52 cm there was no difference between cases 1 to 4 and cases 5 to 10 Birthweights were in normal range 3 610 ± 150 g for boys and 3 200 ± 200 g for girls Four children received 10 mg of hydro cortisone per day i e an average dosage be low 50 mg/m² per 24 hours until the age of 1 year and their height remained close to

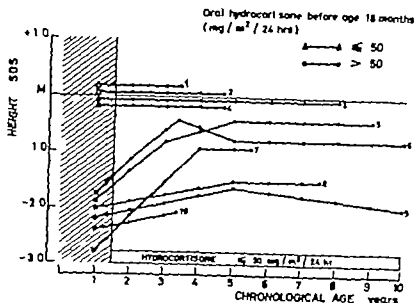


Fig 4 Height distance curves expressed in SDS for 10 patients followed up since early diagnosis The hatched area represents the first 18 months treatment when patients are divided into two groups according to oral hydrocortisone dosages After the age of 18 months all received oral hydrocortisone in dosages equal or inferior to 30 mg/m² per 24 hrs Numbers refer to case numbers reported in Table 1 First recorded heights are not plotted (see text)

mean for age during the whole duration of the study Six children treated initially with excessive dosages (>50 mg/m per 24 hours) had growth retardation greater than 1 8 standard deviations Three children did not show a sufficient catch up growth when treated with dosages equal to or below 30 mg/m per 24 hours capable of maintaining urinary pregnanetriol close to normal values (cases 8 to 10) Three cases (7 to 9) had a good catch up growth increasing their height from 1 5 to 2 5 S D The present growth status of these children is described in Table 1 Among the 4 children who did not undergo early overtreatment (cases 1 to 4) only one (case 2) has a retarded bone age On the other hand among the 6 children overtreated in early life (cases 5 to 10) 3 have bone age retardation higher than 1 year (cases 5 7 9)

Table 1 *Bone age and height SDS in relation to hydrocortisone dosage at last examination in patients treated since early infancy with oral hydrocortisone*

CA chronological age
BA bone age
SDS standard deviation score for height
F hydrocortisone

Case no		Sex	CA (yrs)	BA (yrs)	Height SDS	Dose F (mg/m²/24 hrs)
1	PER	M	3[illegible]	3	0 3	17
2	AUB	F	4[illegible]	3	+0 2	26
3	HUO	M	8	8	0	26
4	RON	F	4[illegible]	5	−0 2	20
5	OUI	M	9	6[illegible]	−0 5	22
6	HUV	F	9[illegible]	10[illegible]	−1	25
7	LOM	F	5[illegible]	3	−1 2	27
8	LED	F	7[illegible]	8	−1 5	26
9	COT	F	10	8	−2	27
10	PAI	F	3[illegible]	2[illegible]	−2	29

Effect of delayed hydrocortisone treatment on linear growth and bone maturation

Three cases are presented in Fig 5 They have been included in group 2 for periods of treatment with hydrocortisone dosages between 30 and 45 mg m per 24 hours Two of them (TRE and BOU) showed a low growth respectively 2 5 cm per year and 2 8 cm per year for long periods If height increments were expressed per year of bone age maturation their values would respectively be 12 cm and 10 cm For that reason linear growth rate appeared to be less affected than bone maturation Patient PER had normal growth and bone maturation rates when dosage was reduced to 20 mg/m per 24 hours

Growth hormone (GH) secretion in treated children

Twelve patients treated with hydrocortisone (16 to 41 mg/m per 24 hours) were studied

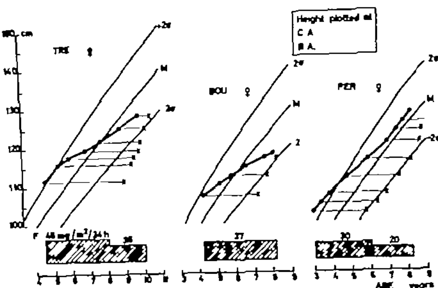


Fig. 5. Effect of delayed oral hydrocortisone treatment on linear growth and bone age in 3 girls with adrenogenital syndrome. (For each one follow up periods were included in group 2 with reduced rates of growth and bone maturation.)

for GH secretion. In 10 cases GH peak values were normal for one or both tests. In cases 5 and 9 the peak value was at the lower limit for normal. There was no correlation with linear growth rate, hydrocortisone dosages or duration of treatment. Hydrocortisone dosages used in this study did not inhibit GH response to arginine or (and) insulin provocative tests.

DISCUSSION

The influence of oral hydrocortisone dosages on growth in children with congenital adrenal hyperplasia has been evaluated by many authors (1, 3, 6, 9, 16, 21). But few longitudinal data are available which permit an approach of the dose response relationship. Most authors agree that overtreatment observed before the age of two years is due to difficulty in management of salt loss or to recurrent infections (3, 9, 16). After the age of three years, dosages between 15 and 36 mg/m² per 24 hours allowed a normal linear growth rate with a slightly reduced skeletal maturation rate which probably indicated a pharmacological effect on cartilage ossification. In spite of a large range in dosages observed most were below the value of 30 mg/m² per 24 hours. A suppressive effect was observed for dosages over 27 mg/m² per 24 hours. There is a definite overlap between both groups. Differences from one subject to another could be due to variable severity of the

Table 2. *Growth hormone response of hydrocortisone treated children to stimulation by arginine and insulin induced hypoglycemia*

Case no.	Plasma GH peak value (ng/ml) ARG test	Plasma GH peak value (ng/ml) INS test	Hydrocortisone (mg/m²/24 hrs)
1 RON	16.2	9.2	40
2 NOG	8.6	16.4	28.5
3 TRE	11	23.5	33
4 LOM	28.6	7.5	36
5 MER F	3.3	3.3	31
6 MER O	1.6	17.8	32
7 MER P	13	10.6	31
8 MED	14.4	11	16
9 PER	4.7	5.4	17
10 MIC J	5.8	18.8	19
11 DRE	9.6	5.8	41
12 MIC P	10.2	11	17
Mean±S.E.M.	10.7±2.0	11.7±1.8	28.5±2.6
Normal (n=11)	10.9±1.7	12.9±3.3	

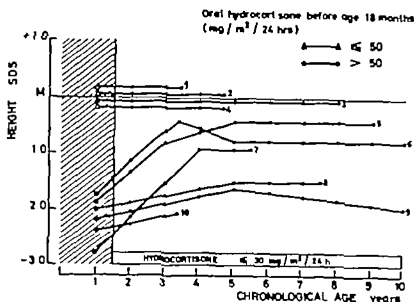


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Case no		Sex	CA (yrs)	BA (yrs)	Height SDS	Dose F (mg/m / 24 hrs)
1	PER	M	$3\frac{8}{12}$	3	+0 3	17
2	AUB	F	$4\frac{1}{12}$	3	+0 2	26
3	HUO	M	8	8	0	26
4	RON	F	$4\frac{9}{12}$	5	−0 2	20
5	GUI	M	9	$6\frac{8}{12}$	−0 5	22
6	HUV	F	$9\frac{9}{12}$	$10\frac{4}{12}$	−1	25
7	LOM	F	$5\frac{8}{12}$	3	−1 2	27
8	LED	F	$7\frac{8}{12}$	8	−1 5	26
9	COT	F	10	8	−2	27
10	PAI	F	$3\frac{3}{12}$	$2\frac{3}{12}$	−2	29

Effect of delayed hydrocortisone treatment on linear growth and bone maturation

Three cases are presented in Fig 5 They have been included in group 2 for periods of treatment with hydrocortisone dosages between 30 and 45 mg/m per 24 hours Two of them (TRE and BOU) showed a low growth respectively 2 5 cm per year and 2 8 cm per year for long periods If height increments were expressed per year of bone age maturation their values would respectively be 12 cm and 10 cm For that reason linear growth rate appeared to be less affected than bone maturation Patient PER had normal growth and bone maturation rates when dosage was reduced to 20 mg/m per 24 hours

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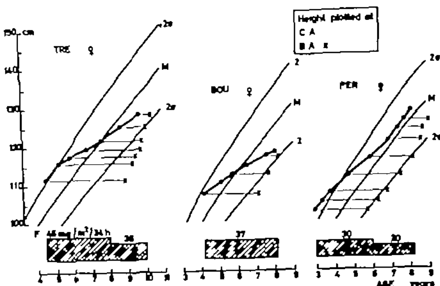


Fig 5 Effect of delayed oral hydrocortisone treatment on linear growth and bone age in 3 girls with adrenogenital syndrome (For each one follow up periods were included in group 2 with reduced rates of growth and bone maturation)

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4 LOM	28 6	7 5	36
5 MER F	5 5	3 3	31
6 MER O	1 6	17 8	32
7 MER P	13	10 6	31
8 MED	14 4	11	16
9 PER	4 7	5 4	17
10 MIC J	5 8	18 8	19
11 DRE	9 6	5 8	41
12 MIC P	10 2	11	17
Mean ± S E M	10 7±2 0	11 7±1 8	28 5±2 6
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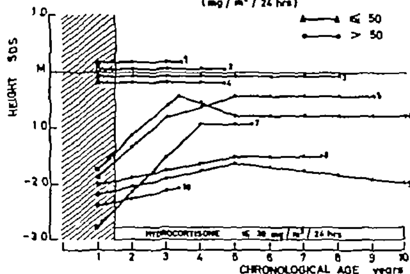


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4 RON	F	4^{9}_{1}	5	−0 2	20
5 GUI	M	9	6^{6}_{1}	−0 5	22
6 HUV	F	9^{9}_{1}	10^{6}_{1}	−1	25
7 LOM	F	5^{6}_{12}	3	−1 2	27
8 LED	F	7^{6}_{12}	8	−1 5	26
9 COT	F	10	8	−2	27
10 PAI	F	3^{8}_{1}	2^{8}_{12}	−2	29

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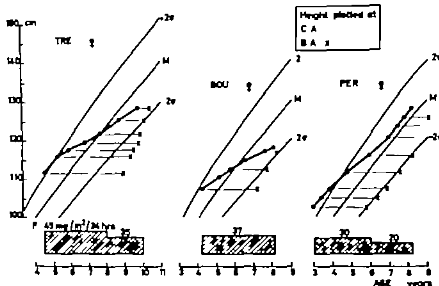


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7 MER P	13	10 6	31
8 MED	14 4	11	16
9 PER	4 7	5 4	17
10 MIC J	5 8	18 8	19
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12 MIC P	10 2	11	17
Mean ± S E M	10 7±2 0	11 7±1 8	28 5±2 6
Normal (n = 11)	10 9±1 7	12 9±3 3	

metabolic defect irregular intestinal absorption or even modifications in the metabolism of orally administered hydrocortisone (2)

The suppressive effect on linear growth and bone maturation is probably due to inhibition of peripheral effect of growth hormone since GH was normally secreted after stimulation in patients receiving up to 41 mg/m per 24 hours of oral hydrocortisone This is in contrast with the GH suppression observed by Stempfel (17) while patients received methyl prednisolone in amounts approximating the normal cortisol production rate When marked retardation of growth and bone maturation was due to dosages of hydrocortisone superior to 50 mg/m per 24 hours before age of 2 years, reducing dosage to the range allowing normal growth after that age did not permit complete catch up in all cases Height distribution yet in a limited number of cases remained below normal mean Hydrocortisone dosages found to allow normal growth rate are perhaps still excessive limiting in some cases potential growth according to genetic possibilities and reducing catch up growth Growth processes would be exquisitely sensitive to hydrocortisone dosages in excess of physiological limits between ages of 3 to 10 years Alternately Mosier (7) showed in experimental studies that cortisone acetate given during eight days to rats in the post weaning period inhibited growth Treated rats showed lack of complete catch up for tail length as well as for body weight Cortisone could permanently damage growth mechanisms and prevent catch up growth Higher doses given to rats produce profound changes in chondrocytes structure and multiplication (5 15) There is however no proof that such conclusions are valid for children treated with hydrocortisone Finally in cases seen late with markedly advanced bone age hydrocortisone dosages between 30 and 45 mg/m per 24 hours could be maintained during several years without noticeable side effects and permitted some linear growth while bone maturation was considerably reduced

SUMMARY

This study was designed to evaluate the effect of various oral hydrocortisone dosages on linear growth and bone maturation in a group of 21 children with congenital adrenal hyperplasia between the age of 3 and 10 years Treatment was monitored on urinary pregnanetriol excretion When considering the individual annual height increment a normal growth rate was achieved with dosages between 15 and 36 mg/m per 24 hours and a reduced growth rate with dosages between 27 and 55 mg/m per 24 hours The same range of dosages was found when bone maturation increments were considered separately In order to study the dose–response relationship the slopes of individual linear growth curves (considered for minimal periods of two years) were correlated with hydrocortisone dosages A significant negative correlation was found which assesses the growth reducing activity of hydrocortisone for dosages up to 45 mg/m per 24 hours Linear growth of children overtreated during early infancy did not show a normal catch up rate in some cases suggesting a permanent damage of growth In all cases growth hormone secretion was in the normal range In late treated children dosages between 30 and 45 mg/m per 24 hours reduced predominantly the skeletal maturation and allowed some linear growth

ACKNOWLEDGEMENTS

Dr Wilhelmi (NIH Bethesda) kindly provided the Human Growth hormone standard for radioimmunoassay

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Key words Congenital adrenal hyperplasia bone maturation growth hydrocortisone

metabolic defect irregular intestinal absorption or even modifications in the metabolism of orally administered hydrocortisone (2)

The suppressive effect on linear growth and bone maturation is probably due to inhibition of peripheral effect of growth hormone since GH was normally secreted after stimulation in patients receiving up to 41 mg/m per 24 hours of oral hydrocortisone This is in contrast with the GH suppression observed by Stempfel (17) while patients received methyl prednisolone in amounts approximating the normal cortisol production rate When marked retardation of growth and bone maturation was due to dosages of hydrocortisone superior to 50 mg/m per 24 hours before age of 2 years, reducing dosage to the range allowing normal growth after that age did not permit complete catch up in all cases Height distribution yet in a limited number of cases remained below normal mean Hydrocortisone dosages found to allow normal growth rate are perhaps still excessive limiting in some cases potential growth according to genetic possibilities and reducing catch up growth Growth processes would be exquisitely sensitive to hydrocortisone dosages in excess of physiological limits between ages of 3 to 10 years Alternately Mosier (7) showed in experimental studies that cortisone acetate given during eight days to rats in the post weaning period inhibited growth Treated rats showed lack of complete catch up for tail length as well as for body weight Cortisone could permanently damage growth mechanisms and prevent catch up growth Higher doses given to rats produce profound changes in chondrocytes structure and multiplication (5 15) There is however no proof that such conclusions are valid for children treated with hydrocortisone Finally in cases seen late with markedly advanced bone age hydrocortisone dosages between 30 and 45 mg/m per 24 hours could be maintained during several years without noticeable side effects and permitted some linear growth while bone maturation was considerably reduced

SUMMARY

This study was designed to evaluate the effect of various oral hydrocortisone dosages on linear growth and bone maturation in a group of 21 children with congenital adrenal hyperplasia between the age of 3 and 10 years Treatment was monitored on urinary pregnanetriol excretion When considering the individual annual height increment a normal growth rate was achieved with dosages between 15 and 36 mg/m per 24 hours and a reduced growth rate with dosages between 27 and 55 mg/m per 24 hours The same range of dosages was found when bone maturation increments were considered separately In order to study the dose–response relationship the slopes of individual linear growth curves (considered for minimal periods of two years) were correlated with hydrocortisone dosages A significant negative correlation was found which assesses the growth reducing activity of hydrocortisone for dosages up to 45 mg/m per 24 hours Linear growth of children overtreated during early infancy did not show a normal catch up rate in some cases suggesting a permanent damage of growth In all cases growth hormone secretion was in the normal range In late treated children dosages between 30 and 45 mg/m per 24 hours reduced predominantly the skeletal maturation and allowed some linear growth

ACKNOWLEDGEMENTS

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GASTRIC LIPOLYSIS OF HUMAN MILK LIPIDS IN INFANTS WITH PYLORIC STENOSIS

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In the suckling rat milk lipids are extensively hydrolysed in the stomach (6) We have found that gastric lipolysis occurs also in the human infant Normally in the infant it would be dif ficult to distinguish gastric from intestinal lipolytic factors since intestinal content can easily regurgitate into the stomach To cir cumvent these difficulties we selected 3 infants with pyloric stenosis for a study of the lipo- lytic activity in the stomach The nature of their disease limits if not excludes, any inter ference from regurgitated intestinal content on the gastric lipolysis proper In all the 3 in fants the clinical diagnosis was later confirmed at operation

MATERIAL AND METHODS

The children were 3–12 weeks old and their body weights ranged between 3 110 and 5 760 g Gastric contents were aspirated by gentle suction through a gastric tube The infants were then given 30 ml of human milk through a formula bottle The milk was heated at 90 for 5 min to inactivate the milk lipases and was at room temperature when given At intervals (Fig 1) samples of gastric content were taken through the tube and the lipid composition was analysed In one infant a prefeeding gastric aspirate was tested for its in vitro lipolytic activity The aspirate was centrifuged in the cold and half of the supernatant was boiled briefly and cooled again Equal volumes of heated and non heated supernatant were incubated at 37 C with an equal volume of human milk in which the lipases had been inactivated by heating The hydrolysis was checked at intervals (Fig 2) The lipids were extracted and separated by thin layer chromatography performed as previously described (6) Glycerol was determined in the lipid fractions by Carlson's method (*1*)

RESULTS

In the experiments *in vivo* the pH of the sam ples ranged from 5 6 to 3 1 With time there was a decrease in triglycerides and a conco- mitant increase in partial glycerides in the gas tric contents (Fig 1 and Table 1) As was pre viously found in the suckling rats (6) the par tial glycerides were mainly diglycerides In Fig 2 the results are shown from the *in vitro* ex periment The pH after mixing was 4 7 As was observed in the *in vivo* experiments the milk triglycerides were hydrolysed by the non heated gastric supernatant forming mainly di glycerides and free fatty acids When milk was incubated under identical conditions but with boiled gastric supernatant there was no hydro- lysis of the milk lipids

DISCUSSION

Since the infants had pyloric stenosis it is un likely that sufficient intestinal content had re gurgitated into the stomach to catalyse the rapid lipolysis observed The low pH values and the relatively high content of diglycerides in the gastric samples also suggest that the hydro lysis was not catalysed by pancreatic or intes

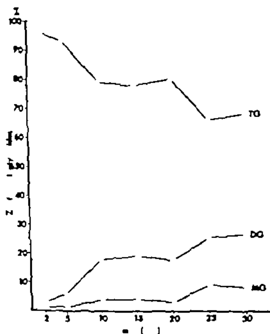


Fig. 1. Composition of lipids in gastric content from an infant with pyloric stenosis (case K. N.) at different intervals after administration of previously heated human milk. The values are expressed in per cent of total glycerides. TG, DG and MG stands for triglyceride, diglyceride and monoglyceride respectively.

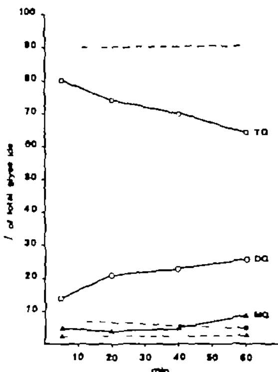


Fig. 2. Hydrolysis *in vitro* of human milk lipids by gastric juice from an infant with pyloric stenosis. The values are expressed in per cent of total glycerides. Filled symbols: heated gastric supernatant. Unfilled symbols: non-heated gastric supernatant. For other abbreviations see Fig. 1.

tinal enzymes (7). Thus some lipase other than of intestinal origin must have catalysed the hydrolysis. This could be the lipase that Cohen et al. (2) have previously described in human gastric juice. They found that the lipase was stable at pH 2, had a lower pH optimum, was rapidly inactivated by trypsin even in the presence of bile acid. The lipase was also decreased in achlorhydric patients. As adult pancreatic lipolytic activity is sufficient for complete hydrolysis of dietary fats, gastric lipolysis might be of minor importance in the adult.

Recently Hamosh & Scow (5) have reported that a lipase is present in the posterior portion of the tongue and in pharyngeal tissues in the rat. This pregastric lipase has a pH-optimum of about 5 and is stable at pH 3 and 37 C for several hours. They also have shown that the main products formed when this enzyme acts *in vitro* on long chain triglycerides are diglycerides and free fatty acids. In calves too a lipase is secreted from the tissues in the pharyngeal region (9). In the human infant we do not yet know which tissue secretes the enzyme. Most likely the origin of the enzymatic activity responsible for the lipolysis observed

Table 1. *Lipids in gastric contents 30 minutes after administration of 30 ml human milk*

Case	Lipid concentration µmol/g		
	TG	DG	MG
P. H.	3.8	1.6	0.7
K. N.	4.1	1.6	0.5
G. L.	7.4	1.4	0.9

The gastric contents were not extracted until several hours after collection. During that time they were kept in a refrigerator. However it is possible that some hydrolysis occurred during the storage. In the two other cases, the gastric contents were kept in ice until extracted not later than 90 minutes after collection.

in the stomach of the infants studied would be analogous to that found in rats and calves If so, the breast milk becomes mixed with the lipase already in the pharynx during swallowing and before the milk is clotted in the stomach

Another lipase is present in human milk (4) and may participate in the digestion of the milk lipids However in the present study the milk had been heated before administration and probably no longer contained any active lipase

In early postnatal life the need for efficient lipid digestion and absorption is high Not only is the amount of food consumed large but it also has a high lipid content However there is doubt whether the infants in the neonatal period are prepared for such a high concentration of lipids in their diet Norman et al (8) have recently shown that the concentration of intestinal bile acids is invariably low which alone may be responsible for incomplete fat absorption in the neonate Furthermore they occasionally found a low concentration of pancreatic lipase

The rates of lipolysis observed in the present study of human infants were such that they could account for approximately one third of the total lipolysis In the suckling rats part of the lipid is made available for rapid absorption and utilization by the gastric lipolysis (3) Also the gastric lipolysis may transform the milk fat droplets to a form which is more readily attacked by the intestinal lipolytic mechanisms

SUMMARY

In gastric contents a potent lipase is present which hydrolyses the milk lipids to mainly diglycerides and free fatty acids This hydrolysis has earlier been demonstrated in rats and now in human infants The gastric lipolysis may facilitate the further digestion of the milk lipids by transformation of the fat droplets to a substrate that is more readily attacked by the pancreatic lipases

ACKNOWLEDGEMENT

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Key words Lipase pregastric fatty acids breast milk feeding

Acta Pædiat Scand 62 523–526 1973

IMPAIRED DELAYED HYPERSENSITIVITY IN SUBACUTE SCLEROSING PANENCEPHALITIS

A KLAIMAN MARION STERNBACH LOTTE RANON M DRUCKER, D GEMINDER and N SADAN

From the Immunological Laboratory Meir Hospital Kfar Saba Tel Aviv University Medical School the Virus Laboratory Ministry of Health Tel Aviv and the Department of Paediatrics Meir Hospital Kfar Saba Israel

Subacute sclerosing panencephalitis (SSPE) is a rare complication of measles and has been reported to follow vaccination with the live virus measles vaccine (9 12) It may follow measles infection or vaccination by a few months but more commonly it appears a few years later (6) Measles antigen has been shown in brain tissue in patients with SSPE by a fluorescent staining technique (5) and a measles like virus has been isolated from the brain tissue (7 9) Measles antibodies are high in the serum and in the cerebro spinal fluid (CSF) (4) SSPE is a disease of child hood which usually presents as a slowly pro gressive deterioration of the intellect and a gradual development of amentia motor sei zures myoclonic jerks spastic paralysis coma and death

Few observations (3 6 8) have been published on the delayed hypersensitive re sponse to measles virus in SSPE Burnet (3) was the first to suggest that in SSPE T lymph ocytes develop specific tolerance to measles antigen while the antibody system remains active

We have examined the immunological re sponsiveness in 2 patients affected by SSPE

CASE HISTORIES

Case 1

N N a 4 year-old boy was admitted to the hospital because of drowsiness, disequilibrium with frequent falls and speech difficulties of 5 days duration He had received live measles vaccine at the age of 2 years and did not have any clinical illness resembling measles On admission the examination revealed a tendency to fall suddenly while standing or sitting the speech was slurred the tendon reflexes were ex aggerated with bilateral ankle clonus Fundoscopy revealed normal eye fundi Measles antibodies titer in serum was 1 256 and in CSF 1 32 (Table 1) The CSF contained 100 mg/100 ml of protein and the electrophoresis disclosed that 38 were gamma globulins The ele troencephalographic examination revealed outbursts of slow waves mixed with sharp waves every 6–10 seconds His neurological condition deteriorated rapidly and ensued in complete amentia within 2 months He died from pneumonia a year later

Case 2

M S a 10 year-old girl was first admitted to the paediatric ward in 1970 at that time th diagnosis of SSPE was made For 3 months she had exhibited in voluntary movements and jerks motor seizures and gradual d terioration of h r intellect At the time of admission th titer of measles antibodies in the serum was 1 2048

Th electro ncephalogram was compatible with SSPE She had had m asles at the age of 18 months

The girl was readmitted to the ward in March 1972 becaus of bilateral bronchopneumonia The neuro logical examination revealed a disoriented and de mented girl unable to talk and incontinent There was a bilateral optic atrophy the limbs were spastic with bila eral extensor plantar reflexes Th titer of measles antibodies in th serum was 1 256 and in the CSF 1 8 (Table 1)

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IMMUNOLOGICAL METHODS

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Table 1 *Immunoglobulin levels in sera and measles antibodies titers in sera and CSF*

Serum (mg/100 ml)			CSF total protein (mg/100 ml)	Measles antibodies titer −1	
IgG	IgM	IgA		Serum	CSF
Case 1					
845 (550–1 490)	67 5 (36–204)[a]	360 (36–232)[a]	100	256	32
Case 2					
920 (720–1 600)[a]	98 (36–256)[a]	295 (48 368)[a]	47	256	8

[a] Normal values for patient s age ±2 S D (1)

and IgA Immunoelectrophoresis was performed in 2 agar Measles antibodies were examined in the serum and in the CSF by the method of hemagglutination inhibition Delayed hypersensitivity was assessed by means of skin tests to an extract of *Candida albicans* (Hollister Stier Laboratories) and PPD the area of induration was measured at 48 hours

Lymphocyte cultures were performed according to the method of Rabinovitz (10) using 20 solution of autologous plasma in Eagle medium The concentration of lymphocytes was 1 × 10 per 1 ml All the examinations were done in triplicates The blastic transformation was counted per 1000 cells The lymphocyte cultures of the 2 patients and 5 controls 4 children who recovered from measles and one after vaccination with live virus vaccine were incubated with 0 1 ml phytohemagglutinin Difco (PHA) for 3 days and for 5 and 7 days with 0 1 ml suspension of killed measles virus (13) and 0 2 ml of 1 10 dilution of the original extract of *Candida albicans* (Hollister Stier Laboratories)

Migration inhibition of peripheral leucocytes (MIT) was don with the buffy coats of the 2 patients and 3 controls using the method of Bendixen & Søborg in capillary tubes (?) Single strength Eagle medium with 10 inactivated horse serum was placed in all the wells The antigens used were either 0 1 ml of killed measles virus or 0 1 ml of *Candida albicans* extract per each well The culture wells were incubated in 5 CO atmosphere at 37 C for 24 hours and the migration index was calculated by dividing the average area of the leukocyte migration in the wells with antigen added to th average area of migration in the wells without antigen All examinations were done in quintuplet and the average of the five examinations was calculated

RESULTS

The peripheral blood counts were normal in the patients The immunoglobulin data are summarized in Table 1

The serum immunoelectrophoresis was normal in both cases No qualitative abnormalities of the immunoglobulins were observed Serum IgA was elevated in case 1 (Table 1) CSF proteins were elevated in case 1 38 • of the proteins were constituted by gammaglobulins they were slightly elevated in case 2 (Table 1)

The delayed hypersensitive response to intradermal injections of *Candida albicans* extract and PPD was good in both cases The 2 patients showed normal lymphocyte transformation with PHA and *Candida albicans* extract but there was no transformation with measles antigen (Table 2)

As the transformations on the 5th and on the 7th day were almost identical, only those on the 5th day are reported

The results of MIT of the peripheral leukocytes of the 2 patients and the 3 controls when tested with killed measles vaccine and *Candida albicans* extracts are presented in Table 3

A migration index of 0 8 or less is considered to be significant The migration of the peripheral blood leukocytes was inhibited in both cases by *Candida extracts* but were totally unaffected by the killed measles virus (Table 3)

DISCUSSION

Subacute sclerosing panencephalitis is a progressive and crippling infection of the central nervous system produced most probably by measles virus Although the disease has been

Table 2. *Percentage of blastic transformation in response to PHA, candida extract and killed measles virus*

	PHA 3 days	Candida 5 days	Measles virus 5 days	Without stimulant
Case 1	87	10	1	1
Case 2	70	7	<0.5	<0.5
Controls	*80–90*	*7–14*	*7–28*	*<0.8*

Table 3. *Results of migration inhibition test (MIT) using candida extract and killed measles virus*

	Case		Control		
Antigen	1	2	1	2	3
Killed measles virus	0.98	1.03	0.50	0.50	0.60
Candida extract	0.80	0.66	0.60	0.34	0.70

recognized for a number of years, only recently a selective abnormality of T lymphocytes has been reported (3, 6, 8) in the presence of normal or even elevated humoral response to the measles virus.

Gerson & Haslam (6) reported in 4 children with SSPE absent or greatly diminished response to intradermal injection of PPD, candida extract, histoplasmin, measles virus and DNCB after previous sensitization. Peripheral blood lymphocyte cultures responded normally to stimulation with PHA and candida extract but showed no reaction to measles virus.

Moulias et al. (8) in their preliminary report stated that none of their 20 patients with SSPE had blastic transformation or inhibition of leukocyte migration when stimulated with measles antigen, though they showed normal response to candida extract and PHA.

One of our patients developed SSPE after vaccination against measles, while the second after infection with the virus. We could demonstrate normal immunoglobulin levels in their sera and a selective derangement of the T lymphocytes to stimulation with killed measles virus, while a normal or nearly normal response to candida extract and PHA was retained.

In Case 2 the lymphoblastic transformation after exposure to PHA was 70%, which is slightly below our normal response of 80 to 90%. Interestingly enough, when this patient's lymphocytes were cultured with PHA in the presence of calf serum instead of the autologous serum the transformation was 86%, well within the normal limits.

Contrary to these observations, Saunders et al. (11) reported a patient with SSPE whose lymphocyte transformation in response to measles antigen was strikingly increased.

The immunological basis of SSPE could be explained by the failure of the T lymphocytes to eliminate the measles virus in the presence of a normal antibody response. Burnet hypothesis (3) that the measles virus is persistently multiplying in the thymus inducing tolerance of T cells to it is an attractive one, although it has not yet been proven. It is highly probable that the persistence of the virus in the brain where it has been demonstrated in SSPE (5, 7, 9) may induce tolerance of the T lymphocytes to it and perpetuate the disease. There is however another possibility that the tolerance of the T lymphocytes enables the virus to persist and survive in the brain.

SUMMARY

Two children with subacute sclerosing panencephalitis showed impaired delayed hypersensitive reaction to killed measles virus in the presence of normal response to candida extract, phytohemagglutinin and PPD. These results suggest that the persistence of the virus in the brain may induce tolerance of the T lymphocytes to it and perpetuate the disease.

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Migration inhibition of peripheral leucocytes (MIT) was don with the buffy coats of the 2 patients and 3 controls using the method of Bendixen & Søborg in capillary tub s (2) Single strength Eagle medium with 10 inactivated horse serum was placed in all the wells Th antigens used were either 0 1 ml of killed measles virus or 0 1 ml of *Candida albicans* extra t per each well The culture wells were incubated in 5 CO atmosphere at 37 C for 24 hours and the migration index was calculated by dividing the average area of the leukocyte migration in the wells with antigen added to th average area of migration in the wells without antigen All examinations were done in quintuplet and the average of the five examinations was calculated

RESULTS

The peripheral blood counts were normal in the patients The immunoglobulin data are summarized in Table 1

The serum immunoelectrophoresis was normal in both cases No qualitative abnormalities of the immunoglobulins were observed Serum IgA was elevated in case 1 (Table 1) CSF proteins were elevated in case 1 38 of the proteins were constituted by gammaglobulins they were slightly elevated in case 2 (Table 1)

The delayed hypersensitive response to intradermal injections of *Candida albicans* extract and PPD was good in both cases The 2 patients showed normal lymphocyte transformation with PHA and *Candida albicans* extract but there was no transformation with measles antigen (Table 2)

As the transformations on the 5th and on the 7th day were almost identical only those on the 5th day are reported

The results of MIT of the peripheral leukocytes of the 2 patients and the 3 controls when tested with killed measles vaccine and *Candida albicans* extracts are presented in Table 3

A migration index of 0 8 or less is considered to be significant The migration of the peripheral blood leukocytes was inhibited in both cases by *Candida extracts* but were totally unaffected by the killed measles virus (Table 3)

DISCUSSION

Subacute sclerosing panencephalitis is a progressive and crippling infection of the central nervous system produced most probably by measles virus Although the disease has been

THE EFFECT OF MATERNAL AGE PARITY AND SOCIAL CLASS ON THE INCIDENCE OF SMALL FOR DATES NEWBORNS

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Small for-dates (S F D) constitute an arbitrarily defined category of growth retarded newborns which is biologically distinct from the group of true premature babies They represent a very small percentage of all newborns but their perinatal mortality is high (374 2‰) (19)

The group of S F D newborns is not etiologically homogeneous (23) Pregnancy complications history of birth of another S F D as well as certain biological social and environmental characteristics have been considered as factors responsible for the birth of a S F D baby (3 5) It would be interesting therefore to further elucidate the possible role of certain important maternal characteristics

The purpose of the present study is to investigate the effect of maternal age parity and social class on the incidence of the S F D newborns

MATERIAL AND METHODS

Our material includes babies born consecutively at the Alexandra Maternity Hospital from 1 8 69 to 1 8 70 Their gestational age was estimated in completed weeks from the first day of the last menstrual period Only mothers who were sure of their dates were included in the present investigation We excluded 478 newborns either because maternal information as to dates were unknown (336 babies) or because of a short gestation (81 newborns) with a gestational age less than 28 weeks Furthermore newborns who belonged to mothers with incomplete information on the studied maternal characteristics (parity social class and age) were also excluded from the study (61 babies) Eventually 4 369 newborns were included in the present investigation Among them 112 having a birth weight of 2 S D or more below the mean for their gestational age were defined as S F D Standard growth curves derived from normal babies born at the same hospital were used (17) Social class classification in the five groups professional or managerial (I) supervisory (II) skilled (III) semi skilled (IV) and unskilled workers (V) was based entirely on paternal occupation (7) For the 39 unmarried mothers occupation of their father was used instead since the majority of Greek women are not employed

The independent effect of each studied factor was estimated by a multiplicative model (18) This assessed the magnitude and the significance of the main effects better than models which assume additivity (6) Thus the χ value as a measure of goodness of fit between the observed numbers of S F D and those predicted by the used model was found low (χ = 18 1 with 22 degrees of freedom) indicating that the used prediction model was adequate

The series of computations were performed by a CDC3300 computer using a program created by J Osborn at the London School of Hygiene and Tropical Medicine

RESULTS

Social class has a very significant effect on the incidence of S F D newborns S F D are very rarely born to mothers belonging to social class I Thus only one of the 132 newborns belonging to social class I was S F D Their incidence increases substantially in social classes II and III Mothers of the lowest social groups (IV and V) give birth to S F D more frequently than mothers belonging to social classes I II or III The distribution of the

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n evaluating fetal growth are maternal age and parity The age of the mother has been substantially associated with her general health and reproductive efficiency (21) It is generally accepted that women attain their highest reproductive potential from the age of 18 to 19 while after the age of 30 this efficiency declines as a consequence of the general ageing process Butler & Alberman (3) noted that in their SFD group with a gestational age of 39–43 weeks there was an excess of low age mothers while MacDonald (11) as well as Ounsted (15) were not able to find any relation between maternal age and intra uterine growth retardation

Our results suggest that very young as well as older mothers have a tendency to an increased incidence of SFD babies as compared with mothers of the 20–30-year old age group Furthermore it is interesting to note that the observed pattern of variation of the incidence of SFD with maternal age does not differ substantially from the pattern of the perinatal mortality (12)

Few and inconclusive reports have been published on the parity effect on the incidence of the SFD newborns (11 13 20) Butler & Alberman (3) reported an excess of primiparae mothers in the group of SFD newborns This was confirmed by Dawkins (4) who noted that primiparity combined with a maternal age greater than 35 should be considered as an important factor predisposing to retarded fetal growth

Our results show that fetal retardation is more common in primiparae This should be attributed to conditions of relative inefficiency of first pregnancies (1) even though the specific underlying mechanisms are not completely understood Baird et al (1) believe that both hormonal and mechanical factors contribute to this inefficiency and they consider first pregnancies as a practice run for subsequent ones These unfavorable factors are possibly responsible also for the reported excessive perinatal mortality of first babies (1 12) Until the underlying mechanism of fetal growth is completely elucidated studies focusing on maternal regulatory factors (16) may possibly help the understanding of some aspects of intra uterine growth

SUMMARY

The incidence of SFD newborns was studied in relation to maternal age parity and social class in a sample of 4 369 consecutive births at the Alexandra Maternity Hospital of Athens The incidence of SFD babies in the lower classes (IV and V) is substantially higher when compared with mothers belonging to social groups I II or III Furthermore the birth of SFD babies is encountered more frequently in very young as well as in women above the age of 30 years Finally the birth of a SFD baby is more frequent in primiparous than multiparous women

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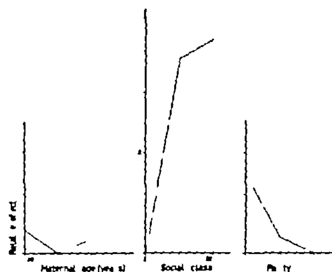


Fig 1 The relative effect of maternal age parity and social class on the incidence of S F D newborns

S F D babies by social class, as well as the relative effect of the social class on the incidence of S F D are shown in Table 1 and Fig 1

The effect of maternal age is also substantial The birth of S F D babies is encountered more frequently in very young as well as very old women Twenty to thirty year old women give birth less frequently to S F D newborns (Table 1 Fig 1) Table 1 and Fig 1 also show that there is a considerable trend for decline of the incidence of S F D with increasing parity but the standard errors of the relative effects are high It is seen that the highest S F D rate is encountered with first births and the lowest with parity over 3

Table 1 *The relative effect of maternal age parity and social class on the incidence of S F D newborns*

Maternal characteristic		No of live newborns	No of S F D	Relative effect	S E of the effect
Parity	0	1 896	65	1 7060	1 0041
	1 + 2	2 212	51	1 1203	0 7021
	3 +	261	6	1 0000	0 7993
Soc class	I	132	1	1 0000	0 3096
	II + III	1 883	50	3 8139	0 2056
	IV + V	2 354	71	4 3547	0 4187
Age	< 20	369	14	1 1796	0 0578
	20 –	2 446	66	1 0000	0 0313
	30 –	1 434	38	1 1080	0 0373
	40 +	120	4	1 4617	0 1280

DISCUSSION

In recent years interest has been focused on those neonates who are born with very low birth weight for their gestational age (2 8) These babies, loosely defined as small for dates evoked increasing interest both as far as their immediate prognosis is concerned as well as for their extra uterine physical and intellectual development (10 22) The group of small for dates comprises babies of lowest birthweight at a given gestational age Two standard deviations from the mean or a weight below the fifth or tenth percentile have been used as cut off points for their identification As Butler & Alberman (3) state babies with a birthweight falling below 2 S D from the mean (about 2° of births) include those with the most severe degree of growth retardation

Undoubtedly the placenta represents an important factor limiting growth of the small for dates It is becoming increasingly clear however that in pregnancies with slow intra uterine fetal growth rates certain maternal factors other than placental insufficiency are more likely to limit the growth potential of the full term baby (14) In some instances racial factors are involved but socioeconomic differences such as for instance those occurring between whites and negroes in the United States have a greater effect on fetal growth than racial ones (9)

According to our results the frequency of S F D newborns is substantially higher in social classes IV and V as compared to social classes I II or III although larger numbers would be necessary for more accurate estimation of its size The factors of the maternal socioeconomic environment are difficult to define Nutrition education living habits health standards etc are difficult to evaluate independently and even to identify and classify We have therefore limited the study of the effect of social class to paternal occupation alone

Some additional aspects which are pertinent

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SUGAR ABSORPTION BY FLAT JEJUNAL MUCOSA

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The normal intestine is divided into two histologically distinct zones the germinative crypts of Lieberkuhn and the absorptive villi (21) In coeliac disease the optical microscopic findings are characterized by short blunt villi longer dilated crypts and abnormal columnar epithelial cells (10 35) Therefore it would seem reasonable to explain the malabsorption in coeliac disease by a decrease in number of the absorptive epithelial villous cells However several findings suggest that such a relationship is more complex 1) It can be assumed that some absorptive function must remain since children with coeliac disease do not normally lose weight except in period of acute diarrhea 2) No significant relationship has been found between histological and clinical absorption studies in either children (15) or in adults (23 31) The gluten free diet brings about striking clinical improvement indisputable evidence of the return of villi is commonly present only in biopsies done several months or more after institution of a gluten free diet (1 37) These differences between clinical and histological findings suggest that several factors might be implicated in malabsorption e g the extent of villous atrophy (15 31) and luminal abnormalities

To obtain further information on the role of jejunal mucosa in malabsorption the report correlates the characteristic activities of epithelial villous cells (disaccharidase activities and maltose and glucose absorptions) with the histology of the same jejunal biopsy specimens

This work was presented in part at the "Réunion de biologie appliquée à la Pédiatrie" Journées Parisiennes de Pédiatrie October 1971

MATERIAL

For ethical reasons jejunal biopsies were performed only on children exhibiting clinical symptoms of malabsorption i e retardation in somatic development and/or decreased fat absorption The biopsy specimens were divided into two groups In group I the villous height was less than 100 µm These fragments were obtained from twenty patients 8 females and 12 males ranging in age from 3 months to 14 years (75% less than 2 years) They all had a diminished fat absorption and a dramatic clinical response to a gluten free diet (increased weight and changed behavior) Since control biopsy after dietary treatment and provocation with gluten have not been made in all the patients this group may reflect different diseases with flat jejunal mucosa (12) In the group II the villous height was greater than 350 µm which is the inferior limit for normal villous height (28 31) These fragments were obtained from 20 patients 9 females and 11 males ranging in age from 3 months to 14 years (75 less than 2 years) They had a similar retardation in height and weight when compared with patients of group I a decreased fat absorption (5 subjects excepted) and often an history of acute diarrhea They were considered as having either cystic fibrosis (2 patients) transient malabsorption syndrome following specific or unspecific diarrhea (13 patients) or somatic retardation with no malabsorption (5 patients) None had evidence of disease involving mucosal damage i e there were no permanent morphologic abnormalities in small bowel X ray studies parasitic infection on stool examination specific histological abnormalities or specific malabsorption syndrome However analysis of duodenal juice for culture and bile salt determination were not performed These jejunal fragments

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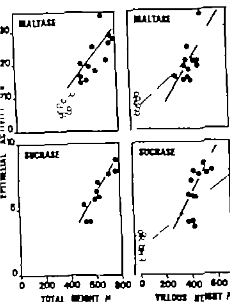


Fig. 1. Relationship between epithelial activity (maltase and sucrase) and histological measurements in μm. 1 unit of disaccharidase activity hydrolyses 1 μmole of disaccharidase per min at 37 C per gram of wet tissue weight (U g). In group II ( ) (villous height higher than 350 μm) the origin ( ) of the extrapolated regression line (—) is not different ($p<0.05$) from zero (maltase = y 0.056 x − 3.2 $p<0.01$ — sucrase = y 0.017 x − 1.0 $p<0.001$). In group I (0) (villous height less than 100 μm the epithelial activity is not zero ($p<0.01$). When epithelial activity is plotted against total height (crypts and villi) the slope of the regression line for group I plus II ( ) (maltase = y 0.053 x 12.3 $p<0.001$—sucrase = y 0.016 x 5.85 $p<0.001$ is not different from the slope of regression line for group II (—) (maltase = y 0.047 x − 7.6 $p<0.01$ — sucrase = y 0.015 x − 3.25 $p<0.01$

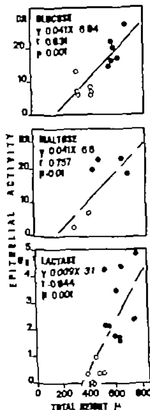


Fig. 2. Relationship between total height and glucose and maltose absorptions and lactase activity. D.R. = Distribution ration is dpm per ml of intracellular fluid over dpm per ml of incubation medium.

Intestinal activity–villous height relationship (Fig. 1)

The functional relationship between villous height and epithelial activity was determined for group II by plotting maltase and sucrase activities against villous height. In this group the correlation coefficient r is significant at 0.01 level for both activities. The origin of the extrapolated regression line is not different from zero ($p<0.05$). Clearly the predicted sucrase or maltase activity would be zero when the villous height is zero. In contrast the observed sucrase or maltase activity in group I is not zero ($p<0.01$).

Intestinal activity–total height relationship (Fig. 1 and 2)

In group I plus II when the intestinal activities were plotted against the total height (from the bottom of the crypt to the villous tip) the correlation coefficient is significant for maltase ($r=0.876$ $p<0.001$) sucrase ($r=0.904$ $p<0.001$) and lactase activities ($r=0.844$ $p<0.001$) and maltose ($r=0.757$ $p<0.01$) and glucose absorption ($r=0.831$ $p<0.001$). Moreover the slopes of the regression lines for sucrase and maltase activities are not significantly different whether determined for group I plus II or for group II. This indicates that the relationship is linear. In contrast there was no significant correlation between lactase activity and total height in group II ($r=0.273$ $p>0.5$). This double determina

Table 1 *Absorptive activity of the biopsy specimens*

Means and standard deviations were measured as usual Since a normal distribution of log values has been described (8) ranges are also mentioned

Villous height		Disaccharidase activity (U × g⁻¹)			Absorption (D R)	
		Maltase	Sucrase	Lactase	Maltose	Glucose
> 350 μm	Mean	21 5	6 5	2 9	21 9	19 0
	S D	5 9	1 6	1 0	2 4	3 8
	Range	14 2–34 5	3 7–8 9	1 6–4 9	18 2–25 0	15 1–27 1
< 100 μm	Mean	7 4	2 2	0 4	6 7	8 7
	S D	2 8	0 7	0 5	3 2	2 2
	Range	3 0–13 9	1 2–3 2	0 0–1 1	2 4–12 0	6 0–13 1
	p	<0 001	<0 001	<0 001	<0 001	<0 001
	Per cent of control activity activity	34	34	13	30	46

might have been obtained from patients suffering from different clinical diseases However they all are within normal histological range (28) and their maltase and sucrase activities are similar to control values (20 22) All these patients (group I and II) were studied while receiving normal (containing gluten and milk) diet without any drug

METHODS

Biopsy technique After a 6 hour fast intestinal tissue was obtained under fluoroscopic control using a Crosby Kugler pediatric capsule All biopsies were obtained from the jejunum near the ligament of Treitz Most often two biopsies were taken at an interval of a few minutes in the same radiologic position Each biopsy specimen was divided to obtain all the required measurements

Histological procedures The tissue was fixed immediately in Bouin s reagent Perpendicular orientation of the mucosal surface during tissue section was determined by the appearance of the exposed crypts A micrometer eyepiece (28) was used to measure 1) total height (T H) i e the distance from the bottom of the crypt to the villous tip 2) villous height (V H) i e from the level of the crypt mouth to the tip These measurements were performed on several sections of each biopsy When the variations were smaller than 100 μm the mean of several villi was used When the variations were higher than 100 μm or when the whole crypts were not seen the biopsy was rejected 3) mitotic index (M I) i e the number of mitoses per hundred cells counted in the crypt

Assays In vitro sugar absorption measurements were done on the remaining material Disaccharidase activities and glucose uptake may be considered as two steps of sugar absorption which may characterize intestinal epithelial cell activity Disaccharidase activities were determined by the method of Dahlqvist (7) 1 unit of disaccharidase activity hydrolyses 1 μmole of disaccharide per minute at 37 C The initial substrate concentration was 0 028 M The results are expressed in term of gram of wet tissue weight The *in vitro* glucose and maltose absorptions were determined by the method of Thier et al (34) The results are expressed as a distribution ratio (D R) which is the disintegrations per minute (dpm) per ml of intracellular fluid over the dpm per ml of incubation medium (the dpm were calculated by the double-channel ratio method) The initial concentrations of glucose and maltose in the incubation medium were 0 1 mM and 0 05 mM respectively The time of incubation was 60 minutes A D R greater than one is presumed to represent uptake in excess of simple diffusion it is a quantitative expression of an active uptake

Statistical studies Each determination was performed independently The means were compared by the Student s t test The regression line was calculated by the least square method The level of significance of the correlation coefficient r was read on the Fisher & Yates tables It was not always possible to determine all the parameters for each biopsy

RESULTS

Mean intestinal activities (Table 1)

The mean disaccharidase activities as well as the mean glucose and maltose absorption is significantly smaller (p 0 001) in group I (V H 100 μm) when compared with group II (V H > 350 μm) Except for lactase activity (group I = 13 of group II) the enzymatic and absorptive activities measured in group I specimens are uniformly about 30% of the corresponding activity in those of group II

II for gly pro dipeptidase (8) 50 , for L-Alanine absorption (9)) In specific congenital malabsorption syndrom the defect is total and specific although the jejunal histology is normal (3 14 24 30 32) The remaining activity of flat muco a suggests the presence of well differentiated absorptive cells in hypertrophic crypts The number of epithelial cells per *unit of length* is constant (28) therefore to estimate the mean activity per cell the relationship between epithelial activity and villous height was studied In group II there is a *significant* relationship between villous height and measured epithelial activity (hydrolysis and uptake) The origin of the extrapolated regression line is not significantly different from *zero Therefore* if flat mucosa is the result of simple villi disappearance the predicted epithelial activity should be zero for a zero height In contrast the measured epithelial activity of flat mucosa (group I) is not zero Two possible explanations for this apparent increase in epithelial activity in flat mucosa are either an increase in activity of the remaining surface cells or the presence of absorptive cells in crypts

The significant relationship between absorptive activity and total height (*i e* crypt and villous cells) suggested an absorptive function for so-called crypts in flat mucosae In *coeliac* disease the optical microscopic findings are characterized by short blunt villi and longer dilated crypts (10 32) Histochemical and electron microscopic studies of the cells of the flat luminal surface have shown striking deviations from the abnormal absorptive epithelium (16 26) Therefore this flat luminal surface does not seem to be the site of the observed epithelial activity Previous studies suggest that the upper part of the apparent crypt in adult idiopathic steatorrhoea has cells with histochemical and cytologic properties of normal villous cells This zone has a rich complement of phosphatases esterase and succinic dehydrogenase which typify absorptive cells (19 26) However these histochemical studies are not quantitative

The linear relationship between maltase or sucrase activities and total height suggests that the mean activity per cell is constant whether the mucosa appears flat or normal A similar relationship was suggested in the rat after partial intestinal resection (11) for *in vivo* glucose absorption Consequently the upper part of the crypt may be the site of the observed epithelial activity

In the rat the disaccharidase activities increase from the crypt to the villous tip (6) A similar distribution was suggested by autoradiographic studies for monosaccharide absorption (21) In other words if the atrophic villi are the result of a simple exfoliation of the higher zone of the villi one would assume that the mean activity per cell is higher *in normal* villi than in flat mucosa In the studied *material* the linear relationship between epithelial activity and total height suggests an increase epithelial activity (hydrolysis and absorption) in flat mucosa

In coeliac disease the mitotic index is increased (26, 37) In our studies there is a *significant relationship* between mitotic index and *total height* However in terms of cell maturation this increase mitotic index may be explained either by a faster cell turnover with a faster rate of protein synthesis or by a longer turnover with a longer period of protein synthesis (35) On the other hand the apparent increased activity of the remaining epithelial cells might be related to a decreased protein turn over

Maltase and sucrase activities and possibly glucose and maltose absorptions seem mainly dependent on the number of differentiated cells In contrast lactase activity seems different There is a greater decrease in lactase activity Although in the subjects studied there is a significant relationship between this activity and the total height it was not possible to find such a significantly relationship either in flat mucosa group or in histologically normal group when studied separately This suggests that lactase activity is dependent on other parameters than cell number (27)

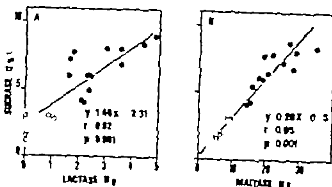


Fig 3 (*A*) Relationship between sucrase activity and lactase activity intercept with vertical axis is significantly different from zero (*B*) Relationship between sucrase activity and maltase activity intercept with vertical axis is not significantly different from zero

tion was not possible for glucose and maltose absorption because the number of measurements was too small The intercepts for the regression lines with the total height are respectively 180 μm for maltose and glucose absorptions 240 μm for maltase and sucrase activities and 340 μm for lactase activity (Fig 2)

Sucrase – lactase relationship (Fig 3)

When lactase activity is zero the sucrase activity is 2 31 U × g⁻¹ In contrast the origin of the sucrase–maltase regression line is not significantly different from zero

Mitotic index – total height relationship (Fig 4)

The mitotic index is higher in group I than in group II ($p<0\,001$) When the villous height is greater than 350 μm the mitotic index has a maximum value of 1 6 In all except 2 flat

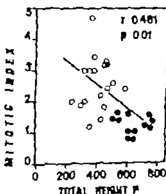


Fig 4 Relationship between mitotic index and total height Mitotic index is the number of mitoses per hundred cells counted in the crypts

mucosae the mitotic index is higher than 1 6 (range 1 2 to 5) When a comparison of mitotic index and total height of all samples (group I plus II) is made there is a significant relationship observed ($r=0\,491$ $p<0\,01$) However when either group I or group II samples are compared no significant relationship appears

DISCUSSION

The patients studied represent two well defined groups as far as histology is concerned In Europe flat jejunal mucosae (group I) are mainly encountered in coeliac disease However the nosology for coeliac disease as defined by the European Society for Paediatric Gastroenterology (12) was not confirmed by histological improvement and subsequently relapse after reintroduction of gluten in all patients All jejunal mucosae in group II had villi higher than 350 μm This has been found to be the inferior limit for normal villous height (28 31)

The decreased disaccharidase activity in flat mucosa has been found by many investigators (2, 8 13 15) The remaining maltase and sucrase activities are slightly higher than those obtained by Dahlqvist et al (8) (maltase 16• sucrase 16 ∘) The maltose and glucose absorptions are decreased to a similar rate These in vitro results are in agreement with in vivo glucose and fructose absorption determinations (17) Similar findings have also been suggested for arbutin (30) The lactase activity was found to be more depressed than the other disaccharidase activities This is in agreement with previous reports (8,25)

The disaccharide absorption involves hydrolysis and transport at the brush border level of the epithelial cells (4) These two processes seem closely related spatially (5) In our studies hydrolysis (maltase activity) transport (glucose absorption) and both activities (maltose absorption) are diminished to the same extent (34 to 46•) in flat mucosa Similarly dipeptidase activity and amino acid absorption are equally affected (from 45•. for gly leu dipeptidase to

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The intercept on the abscissae, of the total height is 100 μm higher with the lactase activity than with the maltase or sucrase activity. Furthermore the persistent sucrase (or maltase) activity for a zero lactase activity is consistent with a different topographic maturation. The lactase activity might appear late in the cell differentiation.

Epithelial absorption and several other factors are involved in the clinical absorption tests. The significant relationship between the studied epithelial activities of the jejunum and total mucosal height contrast with the lack of significant relationship between clinical absorption tests and histologic measurements (15, 23, 31). This suggests that sugar malabsorption in coeliac disease is not a consequence solely of a decreased absorptive (hydrolysis and uptake) epithelial activity. Other factors may also be important.

SUMMARY

To obtain further information on the role of jejunal mucosa in malabsorption, characteristic activities of jejunal epithelial cells (disaccharidase activities and maltose and glucose absorption) were correlated with jejunal histology of 20 biopsy specimens with no villi (flat mucosa, F M (group I)) and 20 biopsy specimens with higher than 350 μm villi (control mucosa, C M (group II). Except for lactase activity (F M = 13% of C M) the enzymatic and absorptive activities measured in F M specimens were uniformly about 30° of the corresponding activity in the C M tissue. An attempt was made to predict maltase and sucrase activities in F M specimens by extrapolating plots of C M activities vs villous height. Measured enzyme activities in F M tissue, however, were significantly higher than the zero activity predicted by such plots. In contrast, the regression lines slopes of maltase or sucrase activities vs total height (crypts and villi) measured in C M specimens were not different from the slopes obtained when data from C M and F M specimens were plotted together. These results suggest (i) the presence of absorptive cells in flat jejunal mucosa, which might be localised in so called crypts, (ii) that a reduction in the number of epithelial cells is not the single mechanism in the origin of flat mucosa, (iii) lactase activity behaves differently than other studied epithelial activities.

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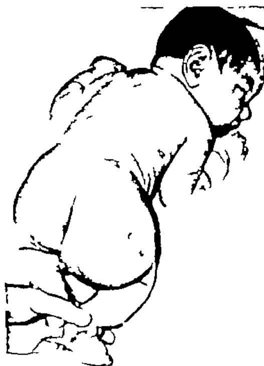


Fig 1 The newborn baby with a right gluteal lymph angioma

well differentiated it had heavily infiltrated the surrounding tissues

12 hours after the operation the wound bled heavily In addition, the left unoperated buttock was swollen and blue indicating a haemorrhage on that side too (Fig 2) Two litres of blood were given within 4 days The dramatic clinical course strongly suggested a coagulation defect and a detailed coagulation analysis was performed on the fourth postoperative day

The fibrinolytic activity of blood from the drain was high and venous blood samples contained fibrinogen fibrin degradation products (FDP) Treatment with AMCA (tranexamic acid Cyclokapron®) in a dose of 0 g 5 intravenously was therefore started and continued for 3 days AMCA was afterwards given by mouth in a dose of 0 5 g 4 This therapy stopped the bleeding The tumour diminished markedly in size

During the healing process (about 6 weeks after the operation) granulations formed from the edges of the wound but regressed on irradiation (1 000 R) of the scar and the wound healed Long term treatment with AMCA in a dose of 1 g twice a day by mouth was started The tumour gradually diminished in size On tentative withdrawal of AMCA the tumour again increased in size and became redder Treatment with AMCA was therefore resumed and the tumour gradually diminished in size

RESULTS OF COAGULATION AND FIBRINOLYTIC STUDIES

The results of the coagulation and fibrinolytic studies are given in Table 1 When first seen at the Coagulation laboratory in November 1969 the platelet count and factor VIII were increased Other factors studied were within normal ranges There were no signs of increased fibrinolytic activity

Coagulation studies were repeated on October 12 1970 the 4th day after the second operation The patient was then bleeding profusely from the operation wound Circulating blood as well as blood drained from the lymph angioma were studied Factor VIII was increased but the platelet count as well as the various constituents of the coagulation system were normal No increased fibrinolytic activity could be demonstrated in the circulating blood which however contained fibrin degradation products (FDP) The blood from the drain possessed high fibrinolytic activity as measured on unheated fibrin plates This blood was also found to contain an extremely high concentration of FDP

Administration of AMCA was followed by disappearance of the fibrinolytic activity from blood drained off The FDP persisted but in lower concentration In the circulating blood the FDP successively fell to nil In the further postoperative course the platelet count and the



Fig 2 1 week after the second operation The buttock is enormously swollen and discoloured

Acta Pædiat Scand 62 538–542 1973

CASE REPORT

FIBRINOLYSIS IN LYMPHANGIOMA

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The bleeding tendency in cases of haemangioma was first described by Kasabach & Merritt (12) in 1940 Since then some 80 cases have been reported Intravascular coagulation (10) and fibrinolysis (11) have been shown to be causes of the bleeding diathesis As far as we know no such coagulation disturbances have ever been observed in patients with lymphangioma

This paper reports profuse bleeding after resection of a giant gluteal lymphangioma The complication was due to fibrinolysis activated by the tumour

METHODS

Platelet and coagulation studies Determinations were made of the platelet count Duke and Ivy bleeding times platelet adhesiveness according to Hellem's whole blood method coagulation time in glass and plastic tubes recalcification time one stage prothrombin time factor VIII factor IX prothrombin factor VII and factor X (Owren's P&P test) and fibrinogen The procedures used have been described earlier (4 14 15)

Fibrinolytic studies The fibrinolytic activity of plasma and resuspended euglobulin precipitate was measured on unheated bovine fibrin plates as described by Nilsson & Olow (15) Plasminogen was measured by an immunochemical method (5) The ethanol gelation test was performed in the way described by Godal et al (6) Fibrin degradation products (FDP) were determined with the immunochemical method of Nilehn (8 13) in serum samples obtained from blood collected with EACA and thrombin

This investigation was supported by grants from the Swedish Medical Research Council (B73 19X 87 09C)

CASE REPORT

The patient a boy born in 1966 was the second child of a healthy woman Pregnancy and delivery had been uncomplicated The baby appeared well at birth except for a pronounced swelling in the right gluteal region (Fig 1)

When the child was 2 1/2 months old surgical exploration and biopsy revealed a benign cavernous lymphangioma An attempt was made to excise the tumour The operation was followed by excessive haemorrhage and wound infection The child was sent home 3 months after the operation but the gluteal and perineal regions were still swollen

A blow against the scar in the right gluteal region when the child was 3 years old was followed by bleeding which persisted for several months The haemoglobin concentration decreased to 6 g/100 ml During this period the scar was invaded by small tortuous vessels and bruised readily Radiotherapy controlled the bruisability but the tumour remained unchanged in size

At 4 years of age the patient was admitted to the University Clinic of Paediatric Surgery Lund At that time the tumour was the size of a grapefruit Angiomatous vessels could be seen in the hard tissue beneath the scar The perineal and left gluteal region was also invaded by soft tumour masses which displaced the anus

Two months later the child was subjected to surgical exploration and biopsy (October 1970) Frozen sections revealed no signs of malignancy The operation was therefore confined to an attempt to reduce the gluteal asymmetry which meant excision of the scar A chunck measuring 10 × 4 × 4 cm was removed by diathermy The blood loss at the operation was negligible

The histological examination showed the characteristics of lymphangioma Though the tumour was

anuary 972	June 1972	Normal range blood
00 000	390 000	150 000–400 000
		1–4
7	6	6–15
8		8–14
22		15–25
14		14–17
65	103	60–140
		60–140
12	97	80–120
00	100	80–120
0 28	0 35	0 24–0 34
		60–140
0	0	0–50
99	59	0–70
Neg		Neg
0	0	0–5

the affected arm to release of activators from the vessels of the angioma Pandolfi & Leander (unpublished data) have recently shown that the endothelium of normal lymph vessels possesses fibrinolytic activity Unfortunately no biopsy specimen was obtained from the tumour for histochemical determination of the fibrinolytic activity in the tissue We nevertheless feel that the most reasonable explanation for the high local fibrinolytic activity in the blood from the tumour in this case was that fibrinolytic activators were released from the endothelium of the vessels of the lymphangioma with consequent activation of the fibrinolytic system The subcutaneous haematolymphangioma described by Gottschalk (7) for example was thought to be caused by trauma with consequent open communication between the blood and the lymph vessels Fibrinolysis or other coagulation defects were not suspected as causal agents Fibrinolysis may nevertheless have been the culprit

Long term treatment with AMCA by mouth was started We thought that inhibition of the fibrinolytic activity in the tumour might facilitate thrombosis of the angioma with consequent reduction in its size In fact the tumour decreased markedly in size as did the intensity of the discoloration It is of course not possible to say anything definitive about the effect of AMCA because spontaneous regression cannot be excluded But since AMCA produced no side-effects we think that it is worth while to try long term treatment with fibrinolytic inhibitors in cases of large lymphangioma and those with a tendency to bleed

The clinical course in these cases stresses the following points Haemorrhage due to fibrinolysis might be expected even in connection with surgical interventions on lymphangiomas Analysis of the drained blood and discharge for fibrinolytic activity and FDP is the diagnostic method of choice Fibrinolytic inhibitors (AMCA) given for a long time may reduce the size of the lymphangioma

SUMMARY

Two operations upon a giant gluteal lymphangioma in a boy were complicated by massive haemorrhage The complication proved to be due to fibrinolysis stimulated by activators released by the tumour The diagnosis was confirmed by the demonstration of high fibrinolytic activity and FDP in blood and discharge drained from the wound The bleeding was effectively controlled by AMCA (tranexamic acid Cyclokapron®) Long term treatment with AMCA was accompanied by further reduction in size of the lymphangioma

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Table 1 *Coagulation analyses*

	November 1969	October 1970 12th Blood	15th[a]	20th	22th Blood	November 1970 4th	January 1971	August 1971
Platelet number per mm³	676 000	318 000			602 000	458 000	394 000	350 000
Bleeding time								
Duke min		2			1	1	2	
Ivy min	8	8			10	8	10	8
Platelet adhesiveness	16	27						
Coagulation time								
Glass min	7	9			14	12	11	8
Plastic min	14	18			30	18	16	12
One stage prothrombin time sec	14	17			25	18	15	14
Factor VIII	195	205			95	68	67	95
Factor IX	160	93			67			
P & P	140	79			49	95	85	90
Factor V	95	80			100	90	98	85
Fibrinogen g/100 ml	0 33	0 33			0 58	0 50	0 39	0 25
Plasminogen		65			95			
Fibrinolytic activity on unheated plates								
Plasma mm²	0	0			0	0	0	0
Resusp euglob prec mm²	28	0			0	10	71	64
Drainage mm²		*750*	0	0				
Ethanol gelation test	Neg	Neg			Neg	Neg	Neg	Neg
FDP μg/ml — blood	0	*40*			*25*	*0*	*0*	*0*
FDP μg/ml — drainage		*5 250*	3 500	*3 500*				

[a] Treatment with AMCA was started October 13th

fibrinogen increased The P & P was low but became normal after administration of Konakion® for 10 days

Laboratory studies during later follow up revealed nothing remarkable and above all no signs of increased fibrinolytic activity

DISCUSSION

The clinic and therapy of lymphangioma have been thoroughly described by Gottschalk (7) Bachmann & Worm (2) and Bill & Sumner (3) The surgical technique has been discussed by Imdahl (11) Our case was initially treated in the conventional way

Already from the very beginning this lymphangioma showed a marked tendency to bleed Routine coagulation analyses of venous blood had not revealed any coagulation defect Observations made at the second operation explained the increased bleeding tendency Samples of blood drained from the operation field showed high fibrinolytic activity and contained an extremely large amount of FDP while the circulating blood showed no increased fibrinolytic activity and FDP in only a low concentration Taken together these findings indicated local fibrinolysis in the lymphangioma The decrease in bleeding and fibrinolytic activator activity in the blood from drain after treatment with AMCA lends further support to the assumption of a fibrinolytic component

The fibrinolytic activators in the body are located in the endothelium of small vessels (16 17) A direct relationship has also been found between the vascularity of a tissue and its fibrinolytic activity (1) Henriksson et al (9) recently described an infant with a giant haemangioma of the right arm and high fibrinolytic activity in the blood of the affected arm Biopsy of the tumour showed high fibrinolytic activity in the walls of the small vessels in the angiomatous tissue They ascribed the increased fibrinolytic activity in the blood in

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		60–140
12	97	80–120
...00	100	80–170
0 .8	0 35	0 24–0 34
		60–140
0	0	0–30
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...eg		Neg
0	0	0–5

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CASE REPORT

PROXIMAL RENAL TUBULAR ACIDOSIS IN VITAMIN D DEFICIENCY RICKETS

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Impairment of the renal regulation of acid base balance in vitamin D deficiency rickets was suggested as early as 1924 (1) This observation was confirmed by studies showing that infants with vitamin D deficiency rickets do not appropriately lower their urinary pH after administration of ammonium chloride (13 14) Participation of parathormone in the physiopathology of this acidification defect was suggested by the demonstration that hydrogen ion excretion is reduced both in hyperparathyroidism (2) and after administration of parathyroid extract (4 9) Recent experimental evidence has been given for a pathogenic role of parathormone in renal proximal tubular acidosis (6 8) This report describes a patient with vitamin D deficiency rickets associated with secondary hyperparathyroidism and proximal tubular acidosis Administration of vitamin D and calcium resulted in the complete correction of all the clinical biochemical and radiographic abnormalities observed in this patient

CASE REPORT

An 8 month-old girl was admitted to hospital because of loss of appetite vomiting and failure to thrive She was a normal full term 3400 g neonate (percentile 10–25) and was fed a skimmed milk without vitamin supplementation At the time of admission she had just been started on a fruit and vegetable diet. Her family history was negative for metabolic diseases.

On physical examination a diagnosis of classical florid rickets was made This diagnosis along with intense secondary hyperparathyroidism was confirmed by X rays and laboratory tests Plasma sodium and magnesium were normal Plasma calcium was 3.9 mEq/l Plasma potassium and phosphate were decreased to 2.9 and 1.1 mEq/l respectively She had a hyperchloraemic metabolic acidosis with plasma bicarbonate concentration of 17.7 mEq/l chloride concentration of 115 mEq/l arterial pH of 7.39 and arterial P_{CO_2} of 29.6 mmHg

Alkaline phosphatase was elevated to 490 IU An anemia was present with Hb of 6.75 g/100 ml Blood urea and creatinine were normal

Routine urinalysis revealed an inappropriate alkaline pH glycosuria, hyperphosphaturia and a generalized aminoaciduria Quantitative analysis showed hyperphosphaturia of 38.4 mg/kg per 24 h Renal concentrating ability and the renal clearance of inulin and PAH were normal as was intravenous urography Because of the excretion of alkaline urine ($pH > 6.5$) in the presence of sustained low plasma bicarbonate concentrations a renal cause for her hyperchloraemic acidosis was suspected On administration of a single oral dose of ammonium chloride 0.1 g/kg urine pH decreased to 5.32 when the plasma bicarbonate concentration was 16.2 mEq/l Excretion of titratable acid amounted to 155.7 µeq/min per 1.73 m^2 Excretion of ammonium ions was 93.3 µeq/min per 1.73 m^2 Twelve days after admission a bicarbonate titration study demonstrated a renal bicarbonate threshold of 16 mEq/l and established a diagnosis of proximal tubular acidosis (Table 1 Fig 2) At this time the low plasma potassium concentration found on admission was not observed Plasma potassium and calcium were normal with values of 3.7 and 4.2 mEq/l respectively Rickets was treated by administration of calcium chloride 1 g daily and of vitamin D 2 000 U daily Proximal renal tubular acidosis was treated by administration of hydrochlorothiazide 10 mg daily Drug therapy resulted in a progressive correction of

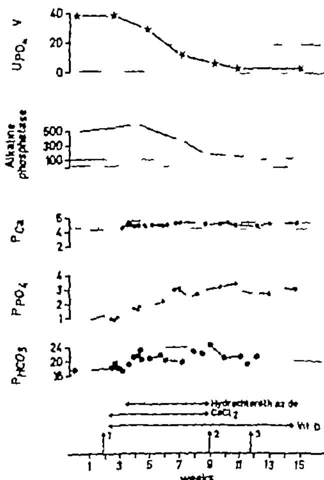


Fig 1 Effect of therapy in a patient with vitamin D deficiency rickets on the plasma concentrations of bicarbonate (P_{HCO_3} mEq/l) phosphate (P_{PO_4} mEq/l) calcium (P_{Ca} mEq/l) alkaline phosphatase (IU) and on the urinary excretion of phosphate (U_{PO_4} V mg/kg/24 h) Shaded areas represent the range of normal values The three arrows 1 2 and 3 on the time scale indicate the time of bicarbonate titration studies

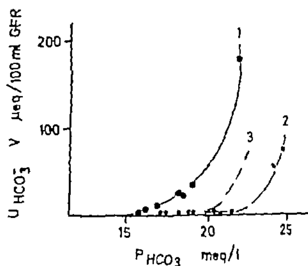


Fig 2 Bicarbonate excretion as a function of the plasma bicarbonate concentration during repeat titration studies in a patient with vitamin D deficiency rickets before treatment (1) while receiving vitamin D calcium chloride and hydrochlorothiazide (2) while receiving vitamin D only (3) Time and conditions at the time of titration studies are described in Table 1 and in Fig 1

the clinical radiographic and chemical abnormalities (Fig 1)

Seven weeks after treatment glycosuria and aminoaciduria had disappeared and phosphate urinary excretion had decreased to 6 mg/kg per 24 h Alkaline phosphatase was approaching normal levels Plasma electrolyte concentrations were within normal limits (Fig 1 Table 1) A second bicarbonate titration study showed a renal bicarbonate threshold of 23 5 mEq/l (Fig 2 Table 1) Correction of the proximal acidification defect could have been due either to healing of the rickets and disappearance of the secondary hyperparathyroidism and/or to the administration of hydrochlorothiazide Thiazides have been shown to increase renal bicarbonate reabsorption presumably through a secondary stimulation of proximal tubular transport due to a depleted extracellular fluid (10) Administration of thiazides was therefore stopped and the patient received vitamin D alone Twenty days later the chemical and radiological abnormalities of rickets and hyperparathyroidism had disappeared Glycosuria and ammoaciduria were absent and urinary phosphate excretion had become normal A third bicarbonate titration study showed a normal bicarbonate threshold of 21 mEq l The patient left the hospital taking a daily dose of 400 U of vitamin D Three months later the patient was clinically well her body weight had increased to 7760 g blood chemistry was normal and there was no radiological signs of rickets

Table 1 *Conditions at the time of the bicarbonate titration studies*

Treatment	Age (weeks)	Weight (kg)	Plasma potassium (mEq/l)	Plasma calcium (mEq/l)	Bicarbonate renal threshold (mEq/l)
Nil	31	5 0	3 7	4 2	16 0
Hydrochlorothiazide 10 mg/day Calcium chloride 1 0 g/day Vitamin D 2000 U/day	38	5 9	3 7	5 2	23 5
Vitamin D 2000 U/day	41	6 3	4 4	4 9	21 0

COMMENTS

This 8 month old girl presented typical vitamin D deficiency rickets and secondary hyperparathyroidism. The diagnosis of vitamin D deficiency rickets was based on clinical, biochemical and radiographic evidence of florid rickets which occurred in a child on a deficient vitamin D diet and responded well to calcium and vitamin D therapy. The presence of secondary hyperparathyroidism was indicated by the low plasma phosphorus, increased phosphate excretion and generalized aminoaciduria (3) as well as by typical radiographic changes in the bones, i.e. metaphyseal subperiostal resorption of bone (5). Rickets and secondary hyperparathyroidism were associated with a proximal renal tubular acidosis. This latter disorder is characterized by bicarbonaturia at normal plasma bicarbonate levels; the defect in bicarbonate reabsorption, and presumably in hydrogen ion secretion, resides in the proximal tubule (11, 12). It is well known that bicarbonate reabsorption is regulated by several factors such as arterial P_{co}, body stores of potassium and relative expansion of extracellular fluid volume. In the present case none of these factors appeared to be involved in the bicarbonate reabsorption defect.

The association of renal tubular acidosis and hyperparathyroidism strongly suggests the possibility of a causal relationship between both factors. Indeed an effect of parathormone on hydrogen ion secretion, and secondarily on bicarbonate reabsorption, has been described (4, 9). Recent observations have definitely incriminated PTH in the pathogenesis of proximal tubular acidosis. Acute administration of PTH to normal subjects induced urinary changes similar to that of proximal RTA (4), while administration of calcium to hypocalcaemic patients with proximal RTA increased bicarbonate reabsorption. This latter effect was reversed by administration of PTH extracts (7). Correction of rickets and hyperparathyroidism in our patient resulted in the normalization of the renal bicarbonate threshold. This result could be related both to the slight increase in the plasma calcium concentration observed after treatment and/or to a decrease in PTH levels. The finding of a supranormal bicarbonate threshold when the patient was receiving hydrochlorothiazide can be explained by the well known effect of this diuretic on bicarbonate reabsorption, presumably via a decrease in ECF volume (10).

Occurrence of proximal renal tubular acidosis in vitamin D deficiency rickets has not been previously described to our knowledge. Winberg & Bergstrom (14) and Whitten (11) reported that the urinary pH of infants with vitamin D deficiency rickets was not lowered appropriately by administration of ammonium chloride, thus suggesting a distal type of tubular acidosis. However since the renal bicarbonate threshold was not determined in these patients, the results do not rule out the possibility of an isolated proximal acidification defect rather than a distal one. Further studies are needed to define the exact nature of the acidification defect accompanying vitamin D deficiency rickets.

SUMMARY

An 8 month old girl presented with classical vitamin D deficiency rickets, secondary hyperparathyroidism and hyperchloraemic acidosis. Renal acidification and bicarbonate titration studies showed the patient to have a proximal renal tubular acidosis. Rickets, secondary hyperparathyroidism and proximal tubular acidosis were corrected by administration of calcium and vitamin D. A causal relationship between hyperparathyroidism and renal proximal tubular acidosis is suggested.

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CASE REPORT

AN EMBRACE REFLEX OBSERVED IN A 5 CM HUMAN FETUS

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Several reflexes can be elicited in short gestation and newborn infants—proprioceptive reflexes such as the palmar grasping reflex or more complex patterns of movement such as the embrace or Moro-reflex (5)

Moro (9) described the latter reflex as an eigenartiger Bewegungsreflex elicited by striking both hands against the pillow under the infant s head The same reflex had according to Joppich & Schulte (5) already been described by Magnus & Kleijn in 1912 elicited by a sudden change of the position of the baby s head Different stimuli may be used e g dropping the baby s head tapping the sternum or pinching the epigastrium (7) The reflex may also be elicited by pulling the blanket or as in the present case the towel on which the fetus was lying

Case Presentation

A 34 year old woman who had borne four children was operated upon for ectopic pregnancy 7 weeks from the last menstrual onset She had four weeks before the operation temporarily suffered from pain in the right side of the hypogastrium followed by persistent slight vaginal bleeding

At the operation a fetus with a crown–rump (CR) length of about 5 cm was found floating in blood in the abdominal cavity It had been extruded from the right tube to which it was still attached by the cord. The heart of the fetus was beating and movements of the extremities noted The fetus was laid on one of the sterile towels close to the wound Unintentionally the towel was pulled and this elicited a brisk obvious embrace reflex

The heart of the fetus was still beating when a photograph was taken (Fig 1) A weak embrace reflex could still be elicited

COMMENT

The period since the last menstrual onset (7 weeks) does not agree with the CR length of the fetus A CR length of 45–50 mm indicates an estimated intra uterine life of 9 to 10 post ovulatory weeks (10) and the ectopic pregnancy was therefore probably 2 weeks old when the patient noted her last bleeding

Several reflexes have been observed in fetuses (1 2 3 4 8) but observations of an embrace reflex in small fetuses have not been found in the literature

The observed reflex was in accord with Moro s description of the embrace reflex given in his lecture in 1918 He suggested that the reflex was phylogenetically related to the primitive embrace reflex exemplified by the orang-outang offspring

Several phases of the Moro-reflex have been observed in the upper extremities—spreading of the fingers extention abduction and finally flexion and adduction of the arms Robinson (11) could not elicit the latter phase of the reflex in his youngest patients Our case displays a very early observation of an embrace reflex It was however the embracing which impressed the whole surgical team—indeed so much so that finer details such as

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Fig 1 Human fetus still attached to the placenta by the cord The tube was ruptured on the side facing the fetus The crown-rump length of the fetus was about 5 cm

finger movements were noted Humphrey (4) stated that if elicited a reflex is entirely normal and our case displayed therefore a probable Moro reflex

Several authors have used a neurological examination including electroencephalographic criteria for the assessment of the true gestational age of the neonate (6 11 12) The Moro reflex has been observed in fetuses about 23 postovulatory weeks old The complete reflex has been elicited at 27 weeks The reflex may well be elicited at an earlier stage of fetal development as illustrated by our case

SUMMARY

An embrace reflex was elicited in a 5 cm crown-rump length human fetus found at operation for ectopic pregnancy The length of the fetus corresponded to an estimated intrauterine life of nine to ten postovulatory weeks

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CASE REPORT

CONGENITAL PAROXYSMAL TACHYCARDIA—A REPORT OF THREE CASES

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Paroxysmal tachycardia in the newborn is a wellknown entity which is not seen too often but which has dramatic symtoms and is quite easy to treat There are cases known with onset before birth (1 3 6 7 8 10) but they are considered to be rare Lundberg has among his 54 cases of infants 6 where the onset might be before birth (5) Quite recently 8 Swedish cases have been reported of paroxysmal tachycardia with onset in utero (4) We will probably find many more if the attention is brought to the clinical picture and if more registrations of fetal heart rates are made

CASE HISTORIES

Case I Girl born 1967 07 07 Pregnancy essentially normal In week 34 fetal heart rate 240/min Spontaneous labor started in week 37 Fetal heart rate at that time 168/min and normal during the labor BW 4010 g the weight of the placenta not known The baby cried immediately but turned rapidly cyanotic with respiratory difficulties grunting Heart rate over 200 no murmurs She was digitalized intravenously and given a dose of diuretics She was referred to the Childrens Hospital in Goteborg at 12 hours of age At that time she was in distress with generalized edema looking like a child of a diabetic mother or one with an intrauterine infection She was slightly cyanotic breathing rapidly with a grossly enlarged liver ECG showed a supraventricular tachycardia with a heart rate of 230/min (Fig 1) X ray showed a large heart with slightly diminished pulmonary vascularity She was continued on digitalis according to her body weight The heart rate decreased during the first few hours and remained at 120/min from 12 hours on The following day she got another small dose of diuretics She was much improved had lost some edema, loosing weight from 4 020 g to 3 170 g at which time she showed signs of an overdose of digitalis vomiting The dose was adjusted accordingly Next day her ECG was within normal limits but during the next few days it showed a WPW syndrome with signs of atrial strain a posttachycardial ECG (5) Thereafter the ECG was normalized (Fig 1) X ray showed diminishing but not yet quite normal heart size No murmur was heard She was maintained on digitalis to 7 months of age and has been followed up to 16 months No recurrent episodes of tachycardia normal heart size Her ECG has been normal without signs of preexcitation (Fig 1)

Case II Boy born 1972 09 29 Normal pregnancy until fetal heart rate was found to be 240/min at 38 weeks A cesarean section was performed BW 3 570 g The baby was depressed slightly cyanotic with a heart rate of 260/min At suctioning of the nasopharynx at 5 min of age the heart rate converted to 150/min and the baby rapidly improved He kept being rather cyanotic and grunting the liver was enlarged as was the heart No murmur was heard Digitalis and diuretics were given the doses calculated from the body weight Urinary production was high during the first 6 hours the baby voided 110 ml and during the next 24 hours 330 ml He lost weight to 3 100 g This made his dose of digitalis temporarily too high he vomited and had a tendency to bradycardia Serum potassium was normal Heart size returned to normal with normal vascularity Particularly remarkable was in this case the placenta weighing 1 100 g and being quite plethoric and edematous as there had also been a great amount of amniotic fluid

Case III Boy born 1969-06-14 Normal pregnancy In week 36 the fetal heart action was found to be rapid and irregular at the routine check rapidly increasing to 250/min A cesarean section was performed BW 3 090 g placental weight 800 g The

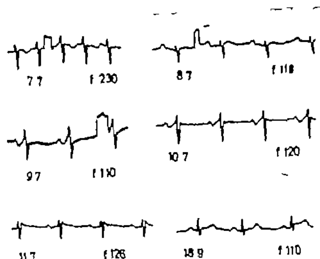


Fig. 1. ECG:s of case I showing the paroxysmal tachycardia, the preexcitation and finally the normal ECG. Lead I. *f*, Heart rate.

baby had a transient extrauterine asphyxia. Heart rate 260/min. Slight cyanosis, grossly enlarged liver. X-ray showed the heart to be enlarged with increased pulmonary vascularity. ECG showed a supraventricular tachycardia with a heart rate of 210/min. The baby was digitalized intravenously and was given a dose of diuretics. The heart rate decreased to 120/min after 6 hours. ECG showed nodal rythm alternating with sinus rythm without any clear WPW syndrome but with temporary signs of atrial strain. He vomited as a sign of overdigitalization when his weight was reduced to 2 560 g and digitalis was temporarily discontinued. Serum electrolytes were normal.

DISCUSSION

Although the first patient did not have a paroxysmal tachycardia during labor, she had clearly had a heart rate of 240/min earlier and the immediate onset of her symtoms after birth makes it reasonable to include this case with those with intrauterine onset. The prenatal onset is quite clear in the two latter cases. There are certainly cases of intrauterine paroxysmal tachycardia that are converted spontaneously to a normal heart rate. They can be misinterpreted as cases of fetal asphyxia leading to a cesarean section. Different types of arrythmia are not uncommon in the fetus which the use of fetal ECG has demonstrated (7, 2). Labor itself with its pressure on the fetal skull and accompanying vagal stimulation may convert an attack of tachycardia (6, 7). Suctioning of the nasopharynx, provoking a reflex vagal stimulation, has terminated the attack in one of our cases.

The two latter cases are especially interesting because of their heavy and edematous placentas, indicating intrauterine cardiac failure. These two cases are quite similar to that of Silber (8). Striking was in case II also the huge amounts of urine.

Digitalization is usually effective in these children. It should be given intravenously and one should be alert as to the possibilities of overdosage when the babies loose weight as their edema disappears. β blocking agents are not given, as these babies are already in cardiac failure.

Their prognosis is not always good: before birth an attack of paroxysmal tachycardia might be fatal (4). But one does not usually see repeated attacks of paroxysmal tachycardia in contrast to the children with a later onset of their symtoms.

The etiology to most of these cases will not be clear, but care should be taken to exclude infections and congenital heart malformations which account for some of them. The preexcitation shown by some 50 % of these patients points to the presence of a bundle of Kent permitting impulses to bypass the AV node and giving a possibility for reciprocal beats.

When a diagnosis of paroxysmal tachycardia

is made before birth labor should be initiated or a cesarean section performed if the fetus is mature The newborn baby should be digitalized which usually leads rapidly to conversion of the tachycardia One should know the possibility of overdosage of digitalis as these babies loose their edema They are usually maintained on digitalis for about half a year

SUMMARY

Three cases of congenital paroxysmal tachycardia are described The babies were in cardiac failure at birth with cyanosis grunting heart and liver enlargement In one case the tachycardia was converted by nasopharynx suctioning in the two by digitalization The three babies lost weight excessively when their edema disappeared and showed temporary signs of overdosage of digitalis All three babies made an uneventful recovery

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ANNOUNCEMENT

SOCIETY FOR EAR NOSE AND THROAT ADVANCES IN CHILDREN

A new interdisciplinary society the *Society for Ear Nose and Throat Advances in Children* (SENTAC) was founded on February 3 1973 The primary purposes of the Society in its designated areas of interest are (1) to promote improvements in the quality of care (2) to stimulate and foster research and (3) encourage and facilitate scientific exchange liaison and coordination among professionals from various concerned disciplines

Elected as initial Directors of the Society were Robert J Ruben M D Albert Einstein College of Medicine President Sylvan E Stool M D University of Pennsylvania School of Medicine Vice President Jack L Paradise M D University of Pittsburgh School of Medicine Secretary Treasurer and Laura A Wilber Ph D Albert Einstein College of Medicine

Applications for membership in SENTAC are invited from otolaryngologists pediatricians audiologists speech pathologists and other interested individuals

Scientific papers are now solicited for presentation at the first Annual Meeting of the Society which will be held in Toronto Ontario at The Hospital for Sick Children on October 9 and 10 1973 Presentations may vary in length from 10 to 45 minutes summaries should be submitted for consideration by July 15

Individuals interested in applying for membership or in additional information concerning the Annual Meeting should write to Jack L Paradise M D Secretary Treasurer SENTAC Children's Hospital of Pittsburgh 125 De Soto Street Pittsburgh Pa 15213



ARVID WALLGREN IN MEMORIAM

Arvid Wallgren the Editor of Acta Paediatrica Scandinavica 1950–1965 died in August 1973 at an age of almost 84 years

Arvid Wallgren was one of the leading pediatricians of our time

After graduation from the Medical School of the University of Uppsala he received postgraduate training in internal medicine and pediatrics At the same time he completed his thesis an experimental study on tuberculosis

At the age of only 33 years Wallgren was appointed Director of the Children's Hospital in Gothenburg which at that time was the largest Children's hospital in Sweden Although this hospital was not a university hospital Wallgren was able to develop an extremely active research program which resulted in a great number of important scientific contributions to pediatrics In 194 Wallgren accepted the call to become Professor of Pediatrics and Chairman of the Department at Karolinska Institute in Stockholm which position he held until he retired in 1956 However Wallgren was very reluctant to accept his well earned *otium cum dignitate* For many years after retirement he held several important positions and then continued to have a great influence not only on pediatrics but also in other medical fields

When in 1950 Arvid Wallgren accepted to become Editor of Acta Paediatrica Scandinavica he was quite aware of the fact that an enormous amount of work would be required of him After a few years of devoted work it was patently obvious that great achievements had been made The journal which earlier had been local to Scandinavia had been transformed into one of the leading international pediatric journals

Whilst Wallgren held office as editor of Acta Paediatrica Scandinavica all the work on the journal was carried out by him alone He not only read evaluated and corrected all manuscripts which were submitted but also dealt with the whole extensive correspondence Since he refused the help of a secretary he also had to type most of the letters which left the Acta office To the Board of our journal it was thus not at all surprising that when Wallgren resigned after 15 years he had to be succeeded not only by another editor but also by a managing editor and a part time secretary After a further four years an assistant managing editor had to join the staff which carried on with the work Arvid Wallgren had earlier coped with himself For those taking over

it was felt to be a great support that Wallgren after his resignation as editor agreed to stay on as co editor for another year and also sit as a member of the Board By this arrangement the editorial staff of the journal has until quite re cently had the great advantage of benefiting from Wallgren s experience and knowledge particularly as regards policy questions All those who were involved in Acta Paediatrica Scandinavica were extremely happy when in 1966 Wallgren accepted the post of honorary editor of our journal

The twenty years Wallgren spent at the Chil dren s Hospital in Gothenburg were filled with intensive scientific activities Of the numerous contributions which were made by him and his associates only a few of the most important can be mentioned here By the introduction of an in tracutaneous method for BCG vaccination he fa cilitated the extensive use of prophylactic inocula tion against tuberculosis He also formulated the principles on which BCG vaccination should be based The results of Wallgren s studies on the natural course of tuberculosis in infancy and child hood still exert a profound influence on our con cepts within this field His thorough research work on the connection between erythema nodosum and primary tuberculosis definitely elucidated a problem which had hitherto remained quite ob scure Important contributions were also made within other fields of pediatrics Wallgren was the first to report the clinical features of acute aseptic meningitis He also presented evidence that acute reticulo endotheliosis and Hand Schuller Christian s disease represent different types of the same underlying disorder In a well know study he demonstrated that pyloric stenosis is not present at birth but develops within the first postnatal weeks

Wallgren s interest in medical research was extremely keen It started early and remained all his life Altogether he published about 400 scien tific papers

Wallgren s scientific contributions made him well know in international pediatrics early in his career Even before the second world war he received a great many invitations from all over the world to present his results His lectures were always highly esteemed Since he had an excellent command of English French and German there was never any language barrier Wallgren was visiting professor at many foreign medical schools Particularly he appreciated the three months he spent at Vanderbilt University Tennessee USA as Abraham Flexner Lecturer in 1949 He re turned home full of enthusiasm and with new ideas which he immediately put into action in his own department

Wallgren was awarded many honorary degrees such as those of the universities of Paris Zurich Santiago Cardiff and Dublin He was an honorary member of more than 20 foreign scientific associa tions He has been a member of the Executive Committee of the International Pediatric Associa tion and of the Executive Board of the Interna tional Children s Center Wallgren served on many UNESCO and UNICEF committees At the age of 70 years he was appointed chairman of the WHO Advisory Committee on Medical Research

Arvid Wallgren was an excellent teacher for undergraduate and postgraduate students It so happened that he was given flowers by the med ical students when they had finished their training in pediatrics Those who have met the rather shy Swedish medical students will understand that such a token of appreciation is quite exceptional Internationally it may be quite unknown that Wallgren in his own country was extremely active in the field of social pediatrics In newspapers magazines and Swedish Radio he repeatedly stressed various views on child health such as the importance of good hygiene correct nutrition and BCG vaccination in the prevention of tuberculosis In Gothenburg he started the first well baby clinics and an outpatient clinic for child psychia try

Wallgren s achievements were enormous They may be explained by an unusually high degree of imagination in combination with a capacity for extremely efficient and hard work He never liked to waste time

Among Swedish friends and friends from all over the world Wallgren was very much ap preciated Their only real complaint was that he could never find as much time to spend with them as they would have liked During the last years before his death Wallgren published his memoirs In this way his friends were extremely happy to have the opportunity to learn more about the man Arvid Wallgren

Rolf Zetterström

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MANNOSIDOSIS
CLINICAL FINE STRUCTURAL AND BIOCHEMICAL FINDINGS IN THREE CASES

SEPPO AUTIO NILS E NORDÉN PER ARNE ÖCKERMAN PAAVO RIEKKINEN* JUHANI RAPOLA and TUULA LOUHIMO

From the Children's Hospital University of Helsinki Helsinki the Children's Castle Helsinki the Department of Neurology University of Turku Turku* Finland and the Department of Clinical Chemistry University Hospital Lund Sweden*

Since the first characterization of mannosidosis in 1967 (16) only two new cases have been published (15) Two further patients published as having an atypical form of mucopolysaccharidosis (12) may in fact have mannosidosis Whether the small number of cases is due to the fact that this disease is extremely rare or to diagnostic difficulties is not known Three new cases recently diagnosed in Finland will be presented here Studies made on these patients may aid in the delineation of the clinical symptoms and signs typical of mannosidosis They also give some insight into the value of various diagnostic procedures and on the classification of the disease

MATERIAL

Case reports[1]

Case 4 PS born 20 9 1961

This patient is the elder of two brothers who are the offspring of asymptomatic noncosanguineous parents The younger brother also suffers from mannosidosis (case 5) No other similar cases are known in the family The pregnancy and the delivery were uncomplicated The boy condition was good immediately after birth and his birth weight was 3 720 g When about 14 weeks old he was admitted to a hospital for enteritis and pneumonia No other abnormalities were observed by the parents during his first year of life At twelve months he spoke a few words but his speech development thereafter appeared delayed Impaired hearing was suspected Audiometry performed at the age of 4 years revealed a loss of hearing of 90 dB at high frequencies The child's speech remained defective and he never spoke whole sentences in spite of a hearing aid

Between the ages of 6 and 9 years he was followed up in the Children's Castle hospital Helsinki He was described as a big boy with a coarse body and face and he was regarded as moderately retarded mentally No obvious impairment of his psychomotor functions was evident during these three years About 30% of the blood lymphocytes were vacuolized The urinary amino acids (high voltage electrophoresis) were normal as well as the urinary glycosaminoglycan (GAG) excretion Th patient was thought to have some metabolic disorder related to the mucopolysaccharidoses and was therefore admitted to the Children's Hospital University of Helsinki

Wh n examined at the age of 9 10/12 years his weight was 35 6 kg height 136 8 cm and head circumference 55 5 cm The bridge of the nose was low the forehead and jaw prominent and the neck somewhat short The chest was broad and there was a slight thoracic kyphosis His hands were small but his fingers were broad and tapering He had thin and relatively long legs The abdomen was protuberant but the liver and spleen were not enlarged He spoke a few indistinct words He had a slightly convergent squint The motor functions were clumsy He was found to be moderately mentally retarded (Terman Merrill method) The neutrophil leucocytes in the peripheral blood and bone marrow contained coarse dark granules The rate of GAG excretion assayed as uronic acid

[1] The cases are numbered according to the order of diagnosis Thus case 1 is the original patient described by Öckerman (16) and cases 2 and 3 were reported by Nordén et al (15)

WHO's classification

it was felt to be a great support that Wallgren after his resignation as editor agreed to stay on as co editor for another year and also sit as a member of the Board By this arrangement the editorial staff of the journal has until quite recently had the great advantage of benefiting from Wallgren s experience and knowledge particularly as regards policy questions All those who were involved in Acta Paediatrica Scandinavica were extremely happy when in 1966 Wallgren accepted the post of honorary editor of our journal

The twenty years Wallgren spent at the Children s Hospital in Gothenburg were filled with intensive scientific activities Of the numerous contributions which were made by him and his associates only a few of the most important can be mentioned here By the introduction of an intracutaneous method for BCG vaccination he facilitated the extensive use of prophylactic inoculation against tuberculosis He also formulated the principles on which BCG vaccination should be based The results of Wallgren s studies on the natural course of tuberculosis in infancy and childhood still exert a profound influence on our concepts within this field His thorough research work on the connection between erythema nodosum and primary tuberculosis definitely elucidated a problem which had hitherto remained quite obscure Important contributions were also made within other fields of pediatrics Wallgren was the first to report the clinical features of acute aseptic meningitis He also presented evidence that acute reticulo endotheliosis and Hand Schuller Christian s disease represent different types of the same underlying disorder In a well know study he demonstrated that pyloric stenosis is not present at birth but develops within the first postnatal weeks

Wallgren s interest in medical research was extremely keen It started early and remained all his life Altogether he published about 400 scientific papers

Wallgren s scientific contributions made him well know in international pediatrics early in his career Even before the second world war he received a great many invitations from all over the world to present his results His lectures were always highly esteemed Since he had an excellent command of English French and German there was never any language barrier Wallgren was visiting professor at many foreign medical schools Particularly he appreciated the three months he spent at Vanderbilt University Tennessee USA as Abraham Flexner Lecturer in 1949 He returned home full of enthusiasm and with new ideas which he immediately put into action in his own department

Wallgren was awarded many honorary degrees such as those of the universities of Paris Zurich Santiago Cardiff and Dublin He was an honorary member of more than 20 foreign scientific associations He has been a member of the Executive Committee of the International Pediatric Association and of the Executive Board of the International Children s Center Wallgren served on many UNESCO and UNICEF committees At the age of 70 years he was appointed chairman of the WHO Advisory Committee on Medical Research

Arvid Wallgren was an excellent teacher for undergraduate and postgraduate students It so happened that he was given flowers by the medical students when they had finished their training in pediatrics Those who have met the rather shy Swedish medical students will understand that such a token of appreciation is quite exceptional Internationally it may be quite unknown that Wallgren in his own country was extremely active in the field of social pediatrics In newspapers magazines and Swedish Radio he repeatedly stressed various views on child health such as the importance of good hygiene correct nutrition and BCG vaccination in the prevention of tuberculosis In Gothenburg he started the first well baby clinics and an outpatient clinic for child psychiatry

Wallgren s achievements were enormous They may be explained by an unusually high degree of imagination in combination with a capacity for extremely efficient and hard work He never liked to waste time

Among Swedish friends and friends from all over the world Wallgren was very much appreciated Their only real complaint was that he could never find as much time to spend with them as they would have liked During the last years before his death Wallgren published his memoirs In this way his friends were extremely happy to have the opportunity to learn more about the man Arvid Wallgren

Rolf Zetterström

spite of adenoidectomy he suffers from recurrent respiratory infections. When he was 2 10/12 years old he was tested for intellectual capacity (Cattel method) and this was then at the level of a one year old child. He is silent, apathetic and indolent. His speech is greatly impaired and his motor functions are clumsy.

METHODS

Histological and electron microscopical studies

Biopsy specimens were taken from the lower edge of the liver. A piece of liver from one patient (case 4) was fixed in buffered 4% formaldehyde for routine paraffin sections. Small pieces of the liver biopsy material were immediately immersed in 2 % glutaraldehyde solution (+4 C) buffered with 0.1 M cacodylate pH 7.3. They were then cut with a razor blade into cubes (about 1 mm³) which were fixed in the same glutaraldehyde solution for 3–6 hours. Subsequently the tissue was rinsed overnight in 7.5% sucrose buffered with 0.1 M cacodylate pH 7.3, changing the solution several times and postfixed in 1% OsO_4 solution with 0.1 M phosphate buffer, pH 7.2. After dehydration with alcohol and propylene oxide the tissue was embedded in Epon 812. Sections (1 µm) stained with toluidine blue were used for orientation. Thin sections stained with uranyl acetate and lead citrate were studied with a Zeiss 9 A electron microscope (accelerating voltage 60 kV).

Enzyme assays

Liver activities of α-mannosidase, acid phosphatase, N-acetyl-β-glucosaminidase, N-acetyl-β-galactosaminidase, α- and β-glucosidase, α- and β-galactosidase, β-glucuronidase and α-fucosidase were assayed by one of us (Raekkinen) using colorimetric methods previously described (22, 23) with a modified incubation medium (0.1 M acetate buffer, pH 4.). Other methods were also used (by Ockerman) for the determination of β-galactosidase, β-glucosidase, α-mannosidase, α-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase and acid phosphatase in liver homogenate (case 4), plasma, urine and white blood cells which were prepared as described earlier (6). β-galactosidase, β-glucosidase and α-mannosidase were assayed fluorometrically as previously described (17, 18, 19). Similar procedures were used for the assay of α-galactosidase (substrate concentration 4.7 mM, acetate buffer pH 4.7). p-nitrophenyl glycosides were used for the assay of acid phosphatase and N-acetyl-β-glucosaminidase.

Carbohydrate assays

Liver homogenate was defatted and hydrolysed (25). Liberated monosaccharides were assayed as their alditol acetate derivatives by gas-liquid chromatography (GLC) (11).

Urinary carbohydrate containing compounds were studied using gel chromatography (Sephadex G 25 fine) of 50 ml urine followed by anthrone and GLC carbohydrate assay as described by Nordén et al. (14).

Table 1. *Anamnestic (A) and clinical (B) data in 3 cases of mannosidosis*

		Case 4	Case 5	Case 6
A	Sex	Male	Male	Male
	Birth date	20 9 61	18 3 68	10 5 67
	Prenatal disorders	−	+	−
	Perinatal disorders	−	−	+
	Delayed early motor development	±	±	+
	Delayed development of speech	+	−	+
	Recurrent infections	+ +	+	+ +
B	Mental retardation	+ +	±	+ +
	Impaired speech	+ +	±	+ +
	Coarse face	+	±	±
	Motor clumsiness	+	±	+
	Cataract	−	+	+
	Testicular hydrocele	−	+	+
	Umbilical hernia	−	+	+
	Hearing defect	+	−	?

Symbols: − = negative, ± = mild, + = moderate, + + = severe, ? = uncertain.

RESULTS

Clinical findings

The most important clinical findings are presented in Table 1. Findings common to all three patients were mental retardation, impaired speech, slightly coarse facial features and clumsy motor functions. Other abnormalities included wheel-like lens opacities in two of the patients, testicular hydrocele in two, umbilical hernia in two and a marked loss of hearing in one of the patients. There was also an unusually strong liability to recurrent infections which most often affected the respiratory tracts. Characteristics of the face and body are shown in Fig. 1.

The main laboratory and radiological observations are presented in Table 2. Vacuolized blood lymphocytes from 20 to 90 % of the cells counted were observed in all cases whenever checked. The vacuoles exhibited sharp margins. They occurred in groups, often surrounding the nucleus. They were also found in lymphoid cells in the bone marrow and there they were also seen in plasma cells. Another haematological abnormality was the presence of coarse dark granules in the polymorphonuclear cells

was 9 7 (carbazole method) 9 4 (orcinol method) mg per 24 hours which was at the upper limit of the normal range The uronic acid/creatinine ratio was 19 8

Radiologically the calvarium was abnormally thick The vertebral bodies especially at the thoraco lumbar border showed osteochondrotic changes The cortex of the long bones was thin with poor trabeculation

A liver biopsy specimen was obtained Electron microscopical (EM) data supported the existence of a storage disease and the diagnosis of mannosidosis was made by enzyme and carbohydrate assays (see below) The patient often suffered from respiratory infections and adenoidectomy was therefore performed recently He is living at home He is quiet and peaceful but easily becomes angry for trivial reasons

Case 5 AS born 18 3 1965

This patient is the younger brother of case 4 There was an imminent miscarriage during the third month of pregnancy He was born 2 weeks before term The delivery was normal His immediate condition was good His birth weight was 3 300 g At three months of age he was operated on for a bilateral testicular hydrocele He was rolling over at 4 months sitting unsupported at 9 months and walking without support at 16 months He spoke a few words at 9 months of age and sentences at 2 years He had recurrent respiratory infections during his first year of life These often recurred during the next years

Vacuolized lymphocytes were first observed at the age of 6 months As tested by the Cattel method at 15 months of age his developmental age was 15–16 months Later a slightly unusual facial appearance and wheel like lens opacities were observed at the Children s Castle Helsinki

The patient was admitted 3 1/2 years old to the Children s Hospital University of Helsinki His weight was 14 2 kg height 95 cm and head circumference 53 cm He had a slightly prominent forehead a low bridge of the nose and prominent ears He spoke short sentences but his speech was somewhat indistinct Other clinical abnormalities observed were a somewhat protuberant abdomen an umbilical hernia thin legs muscular hypotonia and slightly exaggerated ankle jerks His mental function was at the two year level (Terman Merrill test) Vacuolized lymphocytes and neutrophil leucocytes with abnormal granulation similar to that seen in his brother were found in the peripheral blood and bone marrow The urinary GAG excretion was 5 6 (carbazole method) 5 8 (orcinol method) mg per 24 hours The uronic acid/creatinine ratio was 30 7

Radiologically the skull bones were judged to be thick The upper anterior edge of the second lumbar vertebral body was short as compared with the lower anterior edge (lateral projection) The cortex of the long bones appeared thin and their trabeculation was poor

No liver biopsy was performed but the diagnosis of mannosidosis was confirmed by enzyme and carbohydrate assays from blood cells and urine respectively (see below) He is now living at home He is unusually prone to respiratory infections but he is peaceful and co operative

Case 6 HK born 10 5 1967

This patient is the only child of healthy nonconsanguineous patients No similar cases are known in the family The pregnancy was normal The delivery took place 5 weeks before term and his birth weight was 2 680 g He had 9 Apgar scores During the first ? hours after birth he developed symptoms typical of respiratory distress syndrome and was treated with 40 oxygen for 9 days The parents did not notice anything abnormal during his first year of life except for recurrent infections The psychomotor development was slightly delayed He was rolling over to the prone position at 5 months and walking unsupported at 20 months of age His speech development appeared to be retarded He spoke a few indistinct words at the age of 17 months

At a routine follow up control at the age of 9 months 410 out of 500 blood lymphocytes were found to be vacuolized He was admitted to the hospital and there he was thought to be phlegmatic with slight spasticity of the legs There was a bilateral testicular hydrocele The urinary amino acids were normal (high voltage electrophoresis) The GAG excretion was within normal limits (carbazole method) The cell count glucose and protein concentrations of the cerebrospinal fluid were normal The EEG showed no definite abnormality Because of wheel like lenticular opacities he was readmitted to the hospital at the age of 17 months His weight then was 15 5 kg height 86 cm and head circumference 50 cm His facial features were rather unusual and they were described as slightly Hurler like The psychomotor development was a little retarded the motor functions were clumsy and his speech was infantile for his age The muscles were thin and the tendon reflexes were brisk The liver and spleen were not enlarged

The findings at routine urinary and blood tests were normal except for the vacuolized lymphocytes which were seen also in the bone marrow The chromosomal karyotype of peripheral blood lymphocytes was normal The GAG excretion was still within the normal range (carbazol and orcinol methods) The EEG showed a 5–7 c/s theta activity which was mixed with 2 1/2–3 c/s delta waves in the occipital region An audiogram revealed diminished perception of high frequencies but no loss of hearing was evident clinically No histological abnormalities of skin or muscle were found

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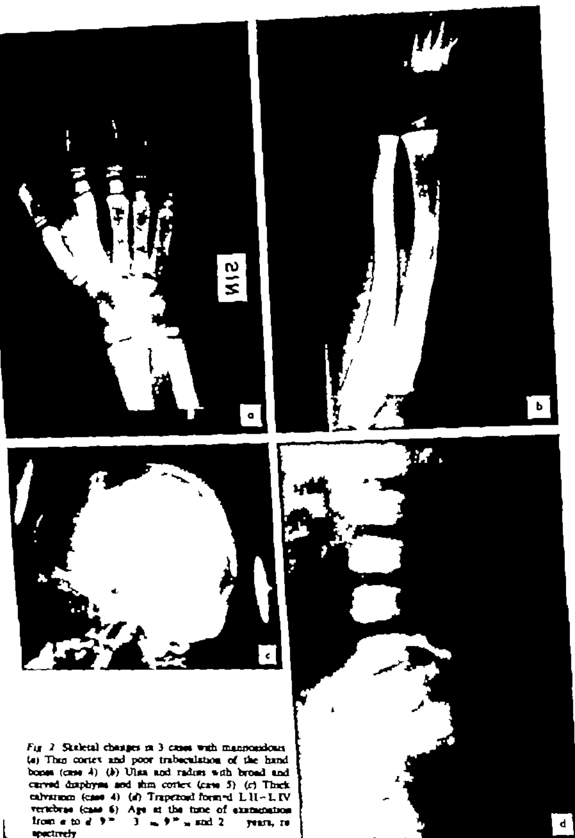


Fig 2 Skeletal changes in 3 cases with mannosidosis (*a*) Thin cortex and poor trabeculation of the hand bones (case 4) (*b*) Ulna and radius with broad and curved diaphyses and thin cortex (case 5) (*c*) Thick calvarium (case 4) (*d*) Trapezoid formed L II – L IV vertebrae (case 6) Age at the time of examination from *a* to *d* 9 [illegible] 3 [illegible], 9 [illegible] and 2 [illegible] years, respectively

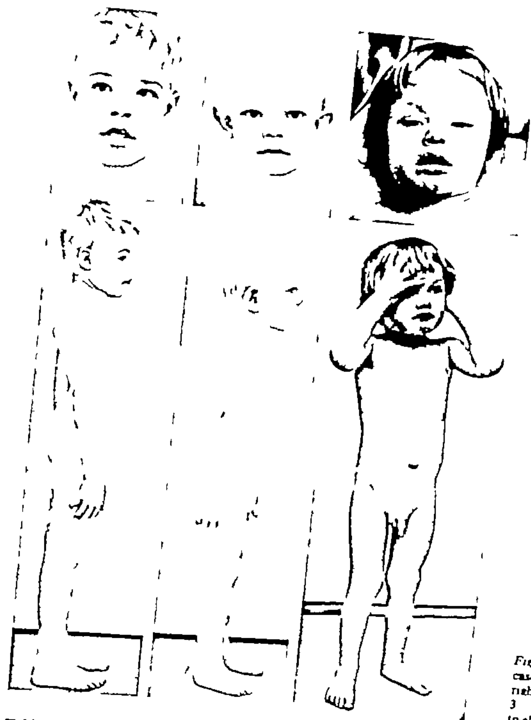


Fig 1 Face and body of cases 4–6 (from left to right) at the age of 9 / 3 and 2 / respectively

Table 2 *Laboratory and radiological findings in 3 cases of mannosidosis*

	Case 4	Case 5	Case 6
Vacuolated lymphocytes in blood	+ +		
Low serum IgG	±	±	±
Mannose rich urinary fraction	+ +	+	
Thick skull	+	+	
Vertebral abnormalities	+	±	
Osteoporotic long bones	+	+	+

Symbols − = negative ± = mild + = moderate + + = marked

in blood as well as in bone marrow Electroencephalography was normal or showed minor unspecific alterations The skeletal changes in common to all 3 patients were osteoporotic long bones with poor trabeculation and a somewhat thick calvaria Hurler like vertebral bodies were seen in the two younger patients while the oldest boy showed vertebral alterations similar to those seen in juvenile osteochondrosis The vertebral abnormalities were most evident in the upper lumbar region (see Fig 2)

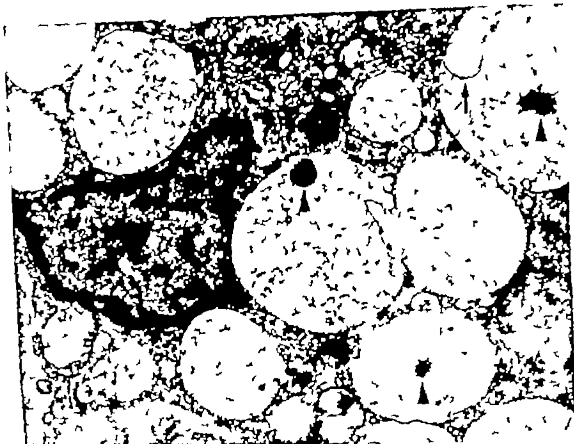


Fig 4 Kupffer's cell with numerous storage vacuoles Two vacuoles in the centrum seem to fuse In addition to the finely granular contents opaque globules (*arrow*) and electron-dense granular aggregates (*arrow heads*) are evident ×20 000

oles in the sinusoids often made it impossible to identify the nature of the cells The size of the vacuoles in these cells varied more than in the hepatocytes from about 0 3 to several μm but their contents appeared similar to those of the hepatocytes vacuoles In several places large vacuoles seemed to have been formed by fusion of the smaller ones (Fig 4)

The bile duct epithelium in the portal tracts showed similar vacuoles but with less reticulogranular material than was seen in the hepatocytes and sinusoidal cells They contained practically no electron opaque globules

Enzyme activities in liver tissue blood plasma blood cells and urine

Liver hydrolase activities in liver biopsy specimens taken from two of the patients (cases 4 and 6) are given in Table 3 The α mannosidase activity was close to the lower limit of the controls while all the other enzyme activities measured were above the range of controls Studies of white blood cells from two of the patients revealed low α mannosidase activities (Table 4) but the plasma activity in these patients was normal (Table 5) Urine from one patient was examined but no deficiency of α mannosidase was found

Carbohydrate assays

Liver The fat free liver extract from one patient (case 4) had a mannose content that was about six times greater than the mean content of 10 controls (Table 6) The galactose and glucose values were within the range of the controls

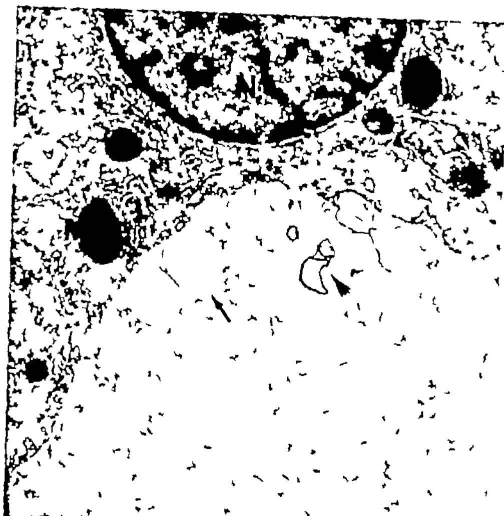


Fig 3 Large storage vacuole in a hepatocyte Finely granular material is dispersed on an electron lucent background Two electron opaque globules are seen close to the inner aspect of the vacuole (*arrow*) Membranous structure is also seen (*arrow head*) *N* = nucleus *M* = mitochondrium × 20 000

Histological and fine structural findings

In paraffin sections of a liver biopsy specimen (case 4) only very sligth histological alterations were observed There was some accumulation of lymphocytes around otherwise normal portal tracts Small cytoplasmic vacuoles were found in most hepatocytes These vacuoles did not stain with PAS or alcian blue The sinusoidal cells appeared normal and there was no increase of connective tissue

The electron microscopical findings in liver specimens were quantitatively and qualitatively almost identical in the two biopsies cases The most striking feature was the presence of storage vacuoles in various types of cells The size of the vacuoles in the hepatocytes varied from 1 5 to 9 0 μm in diameter They were evenly distributed in the cytoplasm and showed no accumulation in the vicinity of the bile capillaries The vacuoles were surrounded by a single membrane and contained a fine reticulogranular material dispersed on an electron lucent background (Fig 3)

Nearly all the vacuoles contained one or more round electron opaque globules usually attached to the inner side of the limiting membrane (Fig 3) These globules rarely exceeded the area of the loose reticulo granular contents Tubular structures electron dense aggregates membrane fragments and so called myelin figures were often seen in the storage material (Figs 3 and 4) Paucity of the peribiliary dense lysosomes was evident No other abnormal cellular components were found and the ordinary cytoplasmic organelles appeared normal

The sinusoids were filled with similar vacuolated material which was also found in the cytoplasm of Kupffer cells and apparently also in the endothelial cells The abundance of vacu

Table 5 *Enzyme activities in plasma*

All values in IU/litre

Enzyme	Case 4	Case 5	Father	Mother
α mannosidase	11	12	11	12
β-galactosidase	0 21	0 44	0 21	0 22
β glucuronidase	1	46	25	27
N acetyl β glucosaminidase	23	4	11	17
α-galactosidase	2 5	2.6	3 0	2 1
β-glucosidase	0 0012	0 0079	0 0012	0 0008

Table 6 *Hexoses in liver tissue*

All figures given as per cent of dry weight

	Mannose	Fucose	Galactose	Glucose
Controls (n = 10)	0 48 (0 21–0 74)	—	0 72 (0 08–0 39)	5 1 (0 21 10 7)
Case 4	3 0	0 70	0 38	8 0

Combined mean values (range) from Öckerman (16) and Hultberg et al (7)

oles were similar to those seen in the mucopolysaccharidoses but in addition there was an extensive affection of the sinusoidal cells

It is remarkable that no definite abnormality of the liver α mannosidase activity was evident It is known that there may be residual activity of this enzyme in mannosidosis even when storage mannosides are used as substrates (5) Thus an assay of α mannosidase alone in suspected cases might lead to a wrong conclusion However if other acid hydrolases are measured concomitantly more definite conclusions can be drawn In the 2 patients whose liver was investigated biochemically α mannosidase was the only activity which remained below the mean activity of the controls while other acid hydrolase activities were high

Studies on enzyme activities in white blood cells suggest that these also may be suitable for diagnostic purposes However α mannosidase has been found to be fairly labile in white blood cells (6) Therefore great caution should be exercised on interpretation of these results The enzyme assays on plasma and urine seem to be of no value for the diagnosis of mannosidosis A possible explanation to this is the existence of three forms of α mannosidase (A B and C) (4) In mannosidosis liver only forms A and B are deficient The most low molecular form C is not deficient and an increased activity of this form in plasma and urine might mask a deficiency of form A and B

The results of the carbohydrate analyses seem to be very clear-cut from a diagnostic point of view The extremely high mannose contents in the liver of case 4 are in agreement with the findings in case 1 (16 20) Urinary mannose containing substances were excreted in abnormally large amounts in cases 4–6 These findings are in close agreement with those seen in cases 2–3 (15) Urine is easier to

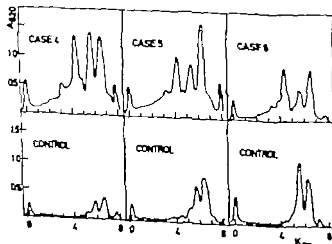


Fig 5 Urinary gel chromatographic fractions assayed by the anthrone method (A_{nm}) Three of the ten controls are shown

Table 3 *Enzyme activities in the liver of 2 cases of mannosidosis*[a]

Enzyme	Case 4	Case 6
α mannosidase	43 (44)	36
Acid phosphatase	254 (173)	246
N acetyl β glucosaminidase	440 (677)	261
N acetyl β galactosaminidase	608	339
α glucosidase	531	392
β glucosidase	133 (188)	150
α galactosidase		300
β galactosidase	417 (271)	298
β glucuronidase	455 (349)	490
α fucosidase	500 (548)	358

[a] The activities are expressed as percentages (mean activity of controls = 100) Öckerman s results are seen within parentheses For controls see ref 13 and 22

Urine Anthrone assay of the gel chromatographic urinary fractions from the 3 patients repeatedly showed an abnormal pattern (Fig 5) not observed in 10 healthy controls (4–10 years old) The characteristic features of the abnormal pattern were high values in many fractions (K_a 0 2–0 6) forming a distinct peak at K = 0 42–0 44

GLC carbohydrate assay of individual gel chromatographic fractions with different K values was performed (Table 7) All three patients had very high mannose values at K 0 33 and 0 42–0 44 as compared with the controls The other values were either within or showed a relatively moderate deviation from the range of controls

The total urinary excretion of mannose containing compounds with K_a less than 0 60 was about 16- to 63 fold higher in the patients than the mean value of the controls (Table 8)

DISCUSSION

Since the number of patients is small it is difficult to draw definite conclusions about the specificity of the symptoms and signs If known data from the other 3 patients with mannosidosis (9 15) are also considered the most constant anamnestic and clinical findings seem to be somewhat coarse facial features mental retardation and liability to recurrent infections A motor clumsiness and speech abnormality may also be typical of mannosidosis The patients displayed several features that are also seen in the mucopolysaccharidoses as well as in aspartylglycosaminuria (AGU) The latter condition is an enzyme deficiency disease recently described (1 8)

Vacuoles lined by a single membrane and containing loose reticulogranular material and round electron opaque globules were first described in Hurler s syndrome (3 10 24) Very similar EM pictures have been observed in other mucopolysaccharidoses and some related disorders (2) This was also the most prominent ultrastructural feature in the present cases Our EM findings were the same as those in the previous ultrastructural investigation of mannosidosis (21) At the present time a differential diagnosis between storage disorders does not seem to be possible with ultrastructural methods alone (2) In our cases the vacu

Table 4 *Enzyme activities in white blood cells*
All values in IU/10⁹ cells

Enzyme	Case 4	Case 5	Father	Mother	Controls[a] (n 10 range)
α mannosidase	0 01	0 003	0 16	0 26	0 20–0 65
Acid phosphatase	29	13	14	23	—
β galactosidase	0 12	0 06	0 06	0 08	0 23–0 54
β glucuronidase	2 4[b]	1 1[b]	1 4[b]	1 6[b]	0 20–0 67
N acetyl β glucosaminidase	11	6 3[c]	5 2	5 4	1 0–6 0
α galactosidase	0 25	0 11	0 15	0 16	—

[a] From ref 6
[b] Substrate used 4 methylumbelliferyl β glucuronide
[c] Substrate used phenolphthaleine β glucuronide

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Table 7 *Carbohydrate assay of individual gel chromatographic fractions*

Results are given as μmoles/ml effluent from the gel chromatographic column per 1 000 ml urine Mean (range) of ten controls is also shown

	K_a value	Mannose	Fucose	Galactose	Glucose	Ribose	Arabinose	Xylose	Inositol
Case 4	0 00	1 44	—	2 52	0 78	—	0 23	0 17	—
	0 22	0 19	0 33	0 28	0 20	0 19	0 00	0 03	—
	0 33	2 77	—	0 11	0 11	0 08	0 08	0 08	0 06
	0 44	9 44	—	0 92	0 92	0 32	0 56	—	—
Case 5	0 00	1 03	1 36	2 03	0 91	0 00	0 00	0 13	0 00
	0 22	0 12	0 18	0 12	0 19	0 09	0 05	0 01	0 02
	0 33	3 04	0 30	0 27	0 49	0 07	0 12	0 05	0 01
	0 42	5 03	—	0 63	1 23	0 15	0 15	0 09	0 00
Case 6	0 00	0 66	0 58	1 47	0 36	0 00	0 03	0 05	0 04
	0 22	0 20	0 00	0 02	0 04	0 04	0 03	0 03	0 01
	0 33	2 41	0 28	0 25	0 26	0 00	0 00	0 00	0 00
	0 44	6 39	1 91	1 14	1 27	0 12	0 15	0 08	0 00
Controls	0 00	0 66	0 82	1 17	0 70	0 17	0 08	0 08	0 04
		(0 29–1 20)	(0 34–1 30)	(0 34–2 48)	(0 28–1 46)	(0 00–1 15)	(0 00–0 49)	(0 01–0 19)	(0 00–0 07)
	0 22	0 07	0 12	0 17	0 22	0 04	0 00	0 01	0 02
		(0 03–0 14)	(0 05–0 19)	(0 08–0 27)	(0 00–0 54)	(0 00–0 19)	(0 00–0 01)	(0 00–0 01)	(0 00–0 06)
	0 33	0 12	0 15	0 09	0 22	0 12	0 01	0 01	0 01
		(0 03–0 18)	(0 00–0 27)	(0 04–0 12)	(0 00–0 46)	(0 00–0 21)	(0 00–0 05)	(0 00–0 03)	(0 00–0 07)
	0 44	0 16	0 29	0 32	0 40	0 04	0 01	0 04	0 02
		(0 07–0 43)	(0 00 0 62)	(0 08–0 56)	(0 00–1 06)	(0 00–0 40)	(0 00–0 05)	(0 00–0 27)	(0 00–0 09)

obtain and handle than liver and white blood cells We therefore believe that the demonstration of an abnormally large quantity of mannose containing urinary substances is an important aid in the diagnosis of mannosidosis

SUMMARY

Three boys 4 5 and 10 years old with psychomotor retardation slightly gargoyle like faces and recurrent infections were found to have vacuolized lymphocytes in the blood and bone marrow as well as diffuse skeletal alterations Electron microscopy of liver biopsy specimens revealed vacuoles presumably representing enlarged lysosomes engorged with storage material The liver α mannosidase activity was somewhat low but not absent while several other acid hydrolases in the liver had very high activities The α mannosidase activity was low also in white blood cells but not in plasma or urine The liver and urine contained very high amounts of mannose rich compounds It is concluded that the patients suffer from mannosidosis

Table 8 *Carbohydrate assay of urinary material with K_a less than 0 60 (Sephadex G 25)*

Values are given as μmoles per litre urine

Sugar	Case 4	Case 5	Case 6	Controls ($n = 10$) M ± S D
Mannose	4 500	3 650	1 160	72 ± 20
Fucose	220	205	270	130 ± 25
Galactose	605	300	230	195 ± 41
Glucose	280	220	195	120 ± 40

ACKNOWLEDGEMENTS

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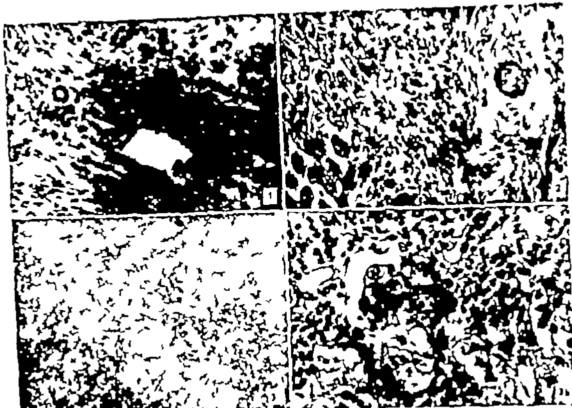


Fig. 1. Chronic persistent hepatitis (type 1). Hematein eosin 300. Clear lobulo-portal junction without piecemeal necrosis. Moderate portal infiltration. Lobular architecture is preserved.

Fig. 2. Chronic progressive hepatitis (type 2 *a*). Hematein eosin 300. Moderate piecemeal necrosis with unclear lobulo-portal junction. Portal fibrosis and inflammatory portal infiltration are moderate.

Fig. 3 a and b. Progressive hepatitis (type 2 *b*). a) Hematein-eosin 40. b) Hematein-eosin 600. Severe piecemeal necrosis. Severe portal and intra lobular fibrosis and inflammatory infiltration. Lobular architecture is profoundly changed.

piecemeal necrosis. Portal fibrosis is severe, as well as portal inflammatory infiltration. Lobular architecture is profoundly changed, with frequent fibrous intralobular septa.

It is on these strict histological criteria that in a group of 33 children first classified as chronic hepatitis, only 16 cases were kept as actual chronic hepatitis; their histological pictures were shown to one of the authors who had defined these histological types in 1967, and he criticized and approved them (17).

RESULTS

Our 16 cases were divided as follows: 3 persistent nonaggressive hepatitis, 6 chronic active hepatitis of the type 2 *a*, and 7 of the type 2 *b*.

As previously reported (4, 7, 9, 11, 16, 19) there was a prevalence of females: 12 girls and 4 boys.

The age of onset of the disease was between 4 and 14 years. However, 16 cases are not enough to allow statistical interpretation. This is why we considered our cases together with those of Grossman (5), Page (12), McLachlan (9) and Willcox (19) (Fig. 4). One can then see that incidence increases from 2 to 15 years, in agreement with adult hepatologists' reports, especially those of Mistilis (11), which state that more than one half of the cases of chronic hepatitis begin between the ages of 10 and 30 years.

Histories of acute hepatitis are difficult to appraise in our 16 patients, even if carefully

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CHRONIC HEPATITIS IN CHILDREN

D ALAGILLE M GAUTIER C HÉROUIN and M HADCHOUEL

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The concept of *chronic hepatitis* first described in adult females by Waldenström (18) in 1950 was applied in children by Good (4) in 1956 Since that date there have been a number of cases reported in children but even now they are not yet as numerous as in adults and great confusion exists in terminology At present chronic hepatitis in children can be divided into two groups chronic meaning a history of hepatitis of more than one year

1 *Persistent hepatitis* (or chronic inactive hepatitis)

2 *Chronic active hepatitis* (lupoid hepatitis active juvenile cirrhosis chronic hepatitis with hyperglobulinemia autoimmune hepatitis chronic viral hepatitis plasma-cell hepatitis)

HISTOLOGICAL CONSIDERATIONS

In more recent years important advances have occurred

1 Total lack of specificity of any clinical or biological feature which has been described by turns as belonging to chronic active hepatitis Such features are no longer considered for diagnosis or prognosis

2 Fundamental importance of the histological findings in differentiating in the group of chronic hepatitis the persistent or nonaggressive hepatitis from chronic active hepatitis with histological changes of aggressivity That is why we have considered only histological features defined in 1967 at the Meeting of the European Association for the Study of the Liver in Goteborg (6) which have been summarized in Table 1 and illustrated by the following examples

Fig 1 concerns chronic persistent hepatitis (histologically of the type 1) taking into account an especially clear lobulo-portal junction without any piecemeal necrosis There is moderate portal infiltration with lymphocytes monocytes or plasma cells and moderate portal fibrosis Moreover lobular architecture is preserved

Fig 2 concerns a case of chronic active hepatitis of mild intensity (histologically of the type 2*a*) of which the more obvious characteristics are a moderate piecemeal necrosis with quite unclear lobulo-portal junction whereas portal fibrosis and inflammatory portal infiltration are moderate Lobular architecture remains either normal or little changed

Fig 3*a* and *b* illustrate severe active hepatitis histologically of the type 2*b* there is great difficulty in seeing lobulo-portal junction owing to a severe

Table 1 *Chronic hepatitis in children histological findings*

	Chronic persistent hepatitis	Chronic aggressive hepatitis mild intensity	Chronic aggressive hepatitis severe intensity
Classification	1	2*a*	2*b*
Lobular architecture	Preserved	Little changed	Changed
Intralobular hepatocellular changes	Focal	Variable	Variable
Portal lymphocyte and plasma-cell infiltration	Moderate	Moderate	Severe
Piecemeal necrosis	0	Moderate	Severe
Intralobular septa			Frequent
Portal fibrosis	Moderate	Moderate	Often severe
Annular fibrosis	0	0	Sometimes

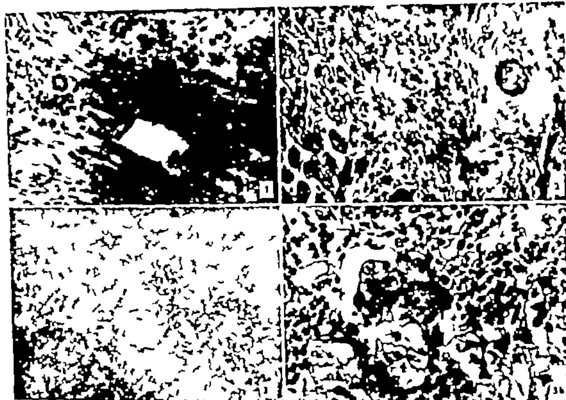


Fig 1 Chronic persistent hepatitis (type 1) Hematein eosin 300 Clear lobulo-portal junction without piecemeal necrosis Moderate portal infiltration Lobular architecture is preserved

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Portal fibrosis	Moderate	Moderate	Often severe
Annular fibrosis	0	0	Sometimes



Fig. 5. Scheme of possibilities of hepatitis evolution

utilized in the latter group with careful supervision of its efficacy by means of clinical biological and overall histological examinations. The few control studies demonstrated a longer survival under corticosteroid therapy with decreased mortality provided early treatment was given during the first year of the disease.

DISCUSSION

Spontaneous recovery in less than 3 months usually occurs in *acute hepatitis*. Yet some cases have a more prolonged evolution which falls into one of 3 types (*Fig. 5*):

(*a*) Cholestasis ending in recovery or perhaps in intrahepatic biliary atresia.

(*b*) Necrosis with persistent or recurrent high levels of serum transaminases and histological persistence of cellular necrosis or alteration.

(*c*) Inflammation with abnormal persistent positive flocculation tests, hyper beta and gamma globulinemia, histological persistence of portal infiltration with monocytes and lymphocytes.

Furthermore these three schematic aspects may be associated. But a few months of follow up shows that in most cases these forms of prolonged hepatitis recover spontaneously without sequelae, generally within 1 year. However a few cases of hepatitis evolve to chronicity, that is beyond 1 year. Differentiation between the two is important because of the possible gravity of prognosis due to cirrhosis.

That is why the present classification of chronic hepatitis (6, 16) with histological criteria represents a practical advance. However changes in liver morphology are very nonspecific and this classification will be utilized only until other etiological and pathogenetic data can be demonstrated.

With reference to etiology our results in the Australia antigen test are disappointing. This is in agreement with Gitnick (3) and Wright's (21) results (Table 3). But we do know that immunodiffusion or electro-immunodiffusion tests, which were used in this study and in many other works (2, 3, 12, 21) are not very sensitive. The tests we are now using—passive hemagglutination, radio-immuno precipitation, radio-immuno assay—will probably increase the frequency of positive results.

It was suggested (2) that humoral and cellular immune reactions have no diagnostic value but are of pathogenetic importance. It is not possible to conclude at present that the reactions are only secondary or that they are of any pathogenetic importance; we hope that immunofluorescence studies of liver cells using specific and anti HB globulins will be more specific and will constitute an important approach to the understanding of the pathogenesis, which will allow improvements in therapy.

With respect to therapy almost all groups use corticosteroid therapy only in cases with histological evidence of aggressivity as we do. However some others (8, 11, 12) have proposed the use of immunosuppressors; however the precise value of these drugs has still to be demonstrated. Moreover the greater tolerance to corticosteroid therapy in children as compared to that in adults is a further argument in favour of this type of treatment, though one must recognize the difficulties involved: necessity for frequent clinical and biological checks, frequent hospitalization for histological studies and ex

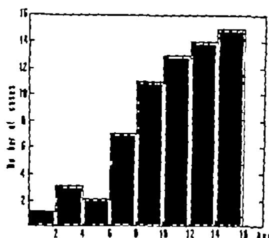


Fig 4 Chronic hepatitis in children Age of onset (personal cases and Grossman Page McLachlan and Wilcox case reports)

restricted to the following criteria jaundice gastro intestinal disturbances impaired general condition contact with a subject who has had hepatitis or characteristic biological changes Only 4 of our 16 cases corresponded to these criteria of which 3 were histologically classified as chronic persistent hepatitis

Australia antigen was positive in only 3 of 9 tested children with immuno diffusion or electro immunodiffusion (Table 2)

The circumstances of discovery varied from evolution of an acute hepatitis or functional symptoms to isolated hepatomegaly Whatever the circumstances of discovery were clinical discussion is almost always restricted by the presence of hepatomegaly which was found in 15 of our cases 7 times it was associated with splenomegaly

Protein abnormalities are of importance hypoalbuminemia hyperglobulinemia usually above 2 g/100 ml during periods of activity they are expressed by positive flocculation tests and increased erythrocyte sedimentation rate high levels of serum transaminases are present during periods of activity in all cases

In general the level of bilirubin in the blood remains normal or slightly increased We found an increase of it in two circumstances at the stage of onset of acute hepatitis or in the course of severe manifestations of hepato cellular failure Schmidt (17) confirmed this observation

One can find positive antinuclear factors, antimitochondrial and anti smooth muscle antibodies High levels of smooth muscle antibodies may be of importance for the diagnostic discussion as distinct from other specific anti organ antibodies Cellular immune reactions may be studied lymphocyte transformation tests leukocyte migration inhibition tests are available

The different types of evolution in our cases are summarized in Table 3 it shows the benign evolution of persistent nonaggressive hepatitis and the extremely severe evolution of chronic active hepatitis with histological aggressivity This is why massive[1] and prolonged corticosteroid therapy has only to be

Table 2 *Chronic hepatitis in children clinical course*

Evolution	No of cases	Classification 1	2a	2b
Death	4	1		3
Recovery	5	2	3	0
Under treatment since 2 years	7	0	3	4
Total	16	3	6	7

Accidental death unrelated to the liver disease

[1] 2 mg of prednisolone per kg body weight and per day during the 3 first months followed by the same dosage every two days

[2] Never less than 2 years and usually between 3 and 5 years

Table 3 *Chronic hepatitis in children detection of Australia antigen*

Immunodiffusion or electro immunodiffusion tests

	No of patients	No of positive results
Gitnick (3)	23	3
Wright (21)	24	6
Personal cases	9	3
Total	56	12

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FAILURE TO THRIVE IN LEBANON

II *An Investigation of the Causes*

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In a previous paper (4) we reported our experience with the use of some simple somatic measurements made in 1 231 Lebanese Moslem Arab children between the ages of 3 and 48 months from low socio-economic families From the relationship of four measurements (weight height head circumference and mid arm circumference) to international standards an Index of Thriving (8) was developed On this basis two contrasting groups of children were distinguished in the 1 231 children

MATERIAL AND METHODS

Th three study areas (rural suburban and urban) were chosen for the possible contrasts which they might afford and also so that they might be representative of different stages of social change in a developing country *Ibe'a* is a Shii Moslem village of about 2 500 inhabitants situated in the south of Lebanon about 70 km from Beirut *Bourj al Barajne* is a suburb of the capital Beirut, about 10 km from th centre of the city and near the International Airport It has a population of about 15 000 inhabitants who are mainly Shii Moslem originating from south Lebanon *Basta* is a down town section of Beirut Here there are more Sunni Moslems than in the other areas

When the Index of Thriving was calculated for the 1 231 children 107 were found to be "Thriving" (Index 0 or 1) and 92 "Failing to Thrive" (Index 9 or more) The first group contained a preponderance of young children below 8 months the usual age of weaning In order to have two comparable groups children below 8 months of age were excluded The study was carried out on data obtained from 53 "Thriving" children in 48 homes and 62 "Failing to Thrive" in 57 homes The two groups were composed of a similar percentage of the population of each of the three areas (Ibe'a 18 9% Thriving 21% Failing to Thrive Bourj al Barajne 62 2% and 58 0 Basta 18 9 and 21 0%) In addition 107 siblings (35 "Thriving" and 70 "Failing to Thrive") were measured and the Index of Thriving calculated

The investigations were conducted in the homes of the study children Thorough medical developmental and anthropometric evaluations were made Separate questionnaires were completed on ( ) socio-economic factors (*b*) household data and (*c*) nutritional beliefs and practices Preliminary accounts of some of these results have b en reported elsewhere (1 3 5 6)

RESULTS

The data obtained have been divided into several categories 1) Family 2) Socio-economic 3) Living conditions 4) Health and 5) Dietary Analysis of the data according to location revealed few statistically significant differences

1) *Family Data*

(i) *Parent s ages* The mean ages of the fathers in the "Thriving" and "Failing to Thrive" groups were not different (35 and 39 years respectively) and of the mothers 29 and 28 years

(ii) *Consanguinity* There was a high consanguinity rate in both groups 28 in the Thriving Group and 29% in the Failing to Thrive Group In the Failing to Thrive Group Basta had 62 cousin marriages and Bourj al Barajne only 19 ($\chi = 7.874$ $p < 0.01$)

(iii) *Age of children* In the Thriving Group

treme difficulty in the decision to stop treat ment

SUMMARY

In a group of 35 children first classified as chronic hepatitis the study of the histological patterns revealed only 16 cases as actual chronic hepatitis 3 persistent nonaggressive hepatitis (type 1) 6 chronic aggressive hepatitis (type 2 *a*) and 7 chronic aggressive hepatitis (type 2 *b*) As previously reported there was a prevalence of females The incidence increases with age A history of acute hepatitis was found in only 4 of the 16 children Australia antigen was positive in only 3 of 9 children tested

Persistent nonactive hepatitis has a benign evolution while chronic active hepatitis with histological aggressivity shows acute rapid evolution It is for this reason that massive and prolonged corticosteroid therapy has to be utilized in the latter group since the few control studies have demonstrated a longer survival

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FAILURE TO THRIVE IN LEBANON

II An Investigation of the Causes

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In a previous paper (4) we reported our experience with the use of some simple somatic measurements made in 1 231 Lebanese Moslem Arab children between the ages of 3 and 48 months from low socio economic families From the relationship of four measurements (weight height head circumference and mid arm circumference) to international standards an "Index of Thriving" (8) was developed On this basis two contrasting groups of children were distinguished in the 1 231 children

MATERIAL AND METHODS

The three study areas (rural suburban and urban) were chosen for the possible contrasts which they might afford and also so that they might be representative of different stages of social change in a developing country *Jba'a* is a Shii Moslem village of about 2 500 inhabitants situated in the south of Lebanon about 70 km from Beirut *Bourj al Barajne* is a suburb of the capital Beirut, about 10 km from the centre of the city and near the International Airport It has a population of about 15 000 inhabitants who are mainly Shii Moslem originating from south Lebanon *Basta* is a down town section of Beirut Here there are more Sunni Moslems than in the other areas

When the Index of Thriving was calculated for the 1 231 children 107 were found to be "Thriving" (Index 0 or 1) and 92 "Failing to Thrive" (Index 9 or more) The first group contained a preponderance of young children below 8 months the usual age of weaning In order to have two comparable groups, children below 8 months of age were excluded The study was carried out on data obtained from 53 "Thriving" children in 46 homes and 62 "Failing to Thrive" in 57 homes The two groups were composed of a similar percentage of the population of each of the three areas (Jba'a 18.9% Thriving 21% Failing to Thrive Bourj al Barajne 62.2 and 58.0 Basta 18.9 and 21.0) In addition 107 siblings (35 "Thriving" and 70 "Failing to Thrive") were measured and the Index of Thriving calculated

The investigations were conducted in the homes of the study children Thorough medical developmental and anthropometric evaluations were made Separate questionnaires were completed on (*a*) socio-economic factors (*b*) household data, and (*c*) nutritional beliefs and practices Preliminary accounts of some of these results have been reported elsewhere (1 3 5 6)

RESULTS

The data obtained have been divided into several categories 1) Family 2) Socio-economic 3) Living conditions 4) Health and 5) Dietary Analysis of the data according to location revealed few statistically significant differences

1) *Family Data*

(i) *Parent's ages* The mean ages of the fathers in the "Thriving" and "Failing to Thrive" groups were not different (35 and 39 years respectively) and of the mothers 29 and 28 years

(ii) *Consanguinity* There was a high consanguinity rate in both groups 28 in the Thriving Group and 29.4 in the Failing to Thrive Group In the Failing to Thrive Group Basta had 62 cousin marriages and Bourj al Barajne only 19 ($\chi^2 = 7.874$ $p < 0.01$)

(iii) *Age of children* In the Thriving Group

Table 1 *Education of parents*

	Thriving (48)		Failing to Thrive (57)	
	Fathers ( )	Mothers ( )	Fathers ( )	Mothers ( )
Koranic*	62 3	32 1	54 8	1 6
Primary	11 3	11 3	4 8	6 5
Secondary	9 4	0	1 7	0
None	17 0	56 6	38 7	91 9

* Able to read and write classical arabic

the mean age was 17 3 months while the Failing to Thrive Group had a mean age of 20 2 months This difference was not significant

(iv) *Sex of children* There were 24 boys and 29 girls among the Thriving and in the Failing to Thrive Group there were 25 boys and 37 girls

(v) *Birth size* Birth weights were unfortunately not available but estimates given by the mothers showed a higher proportion of lighter babies in the Failing to Thrive Group than in the Thriving Group

(vi) *Birth rank* The mean birth rank of the Thriving Group was 5 5 and is not significantly different from that of the Failing to Thrive (mean 6 1)

(vii) *Family size* The average number of children in the Thriving Group families was 4 4 (range 2–14) and in Failing to Thrive families was 5 1 (range 2–10) Large families (more than 4 children) were more common in the Failing to Thrive Group (χ = 7 595 and 0 01 > p > 0 001)

(viii) *Siblings Index of Thriving* The Index of Thriving in the siblings of the Thriving Group (mean 3 6) was significantly lower than that of the Failing to Thrive Group (mean 5 3) (0 02 > p > 0 01), indicating better growth in the former

(ix) *Abortions and sibling deaths* The proportion of the mothers who had had an abortion was greater in the Failing to Thrive (44 6%) than in the Thriving (41 6%) However abortions were more frequent in the Thriving than in the Failing to Thrive Group (18 5% vs 10 8% of pregnancies χ = 7 640 and 0 01 > p > 0 001) The greater number of abortions in the Thriving Group may have been due to induced rather than to spontaneous abortions There were not significantly more deaths (10 3% of pregnancies) among siblings of the Failing to Thrive Group than in the Thriving Group (7 5%)

Table 2 *Distribution of radios television sets and refrigerators*

	Thriving (48)		Failing to Thrive (57)	
	No		No	
Radio	44	87 3	44	78 7
TV	12	24 1	9	16 1
Refrigerators	35	65 8	8	31 1
Refrigerator + Radio or TV	33	63 3	15	27 9
None	1	6 3	8	14 5

2) *Socio-economic Data*

(i) *Education of parents* At all levels a greater proportion of both sexes in the Thriving Group was educated and in both groups fathers were better educated than mothers (Table 1) A higher percentage of fathers (χ = 6 62 and 0 01 > p > 0 001) and mothers (χ = 18 99 and p < 0 001) in the Failing to Thrive Group had no education

(ii) *Occupation of parents* Most of the fathers were employed on the land or as casual semi skilled or unskilled workers in various trades (81 1% in the Thriving Group and 80 2 % in the Failing to Thrive Group) In both groups few mothers admitted to working for people other than their families

(iii) *Income* The mean monthly income per family was significantly higher in the Thriving Group (£ L 242) than in the Failing to Thrive (£ L 213) p < 0 001 One Lebanese pound is about 30 U S cents There were significantly more families with an income in excess of £ L 250 in the Thriving Group (χ = 26 85 p < 0 001) On a per capita basis the difference would be even greater No differences were observed within the same group in the three areas

(vi) *Household possessions* The greatest difference between the groups was in the possession of a refrigerator ($\chi = 17\ 91$ and $p < 0\ 001$) (Table 2) Within the same group the families in Jbeil owned fewer refrigerators than families in the other two areas This may be due to the more conservative attitude of rural communities

3) *Living conditions*

Most of the houses are of concrete construction In both groups more than 60 % of the houses were rented In Jbeil however a higher proportion (75 %) of both groups owned their own houses than in the urban areas

(i) The Thriving Group lived in clean and well kept houses with more rooms than the other group For homes with 3 rooms and more they had a significantly higher percentage ($\chi = 16\ 19$ and $p < 0\ 001$)

(ii) Toilet facilities were usually inside the home in the Thriving Group (95 8 %) and less often so in the Failing to Thrive Group (73 7 %) ($\chi = 9\ 532$ and $0\ 01 > p > 0\ 001$)

(iii) A special bathroom was more often found in the Thriving Group (85 4 %) than in the Failing to Thrive (52 6 %) ($\chi = 12\ 931$ and $p < 0\ 001$)

(iv) Water was available in the homes of the Thriving Group (95 8 %) significantly more often than in those of the Failing to Thrive (73 4 %) ($\chi = 8\ 35$ and $0\ 01 > p > 0\ 001$)

(v) The kitchen was a special room in 97 9 % of the Thriving Group while for 16 % of the Failing to Thrive the food was cooked either outside or in one of the living rooms ($\chi = 5\ 77$ and $0\ 01 < p < 0\ 02$)

Rural homes of both groups were usually larger than urban and general living conditions better

4) *Health Data*

(i) *Family health history* Cardio vascular system disease diabetes and cancer were all more common in the adults of the Thriving Group than in the Failing to Thrive Group though not significantly so

Table 3 *Mean daily calorie and protein intake*

	Thriving (6)		Failing to Thrive (45)	
	calories	protein	calories	protein
No of cases	kcals	g	kcals	g
Mean intake/day	543	18	389	17 9
Range	(260–840	(10 4–39 0)	(16 [illegible]–750)	(7 9–39 0)
% of requirements Age 0–3 months				
above 85	14	25	1	39
below 85	12	1	33	6
% of requirements Age 4–6 months				
above 85	6	24	3	31
below 85	20		42	14

(ii) *Mother's antenatal history* Health during pregnancy did not differ significantly in the mothers of the two groups Morning sickness however was nearly twice as frequent in the Failing to Thrive Group

(iii) *Study child's medical history* Children in the Failing to Thrive Group had a higher prevalence of measles (29 0 % v 26 4 %) and gastro-enteritis (80 7 % v 67 9 %) but only for pertussis (12 9 % v 1 9 %) was the difference significant ($\chi = 4\ 96$ and $0\ 02 < p < 0\ 05$) Upper and lower respiratory infections were more common in the Thriving Group (75 5 % v 61 3 %)

In both groups children experienced equally repeated episodes of both gastro enteritis and upper and lower respiratory infections The estimated duration of illness tended to be shorter in the Thriving Group More of those who had suffered from gastro enteritis for more than 1 month were in the Failing to Thrive Group ($\chi = 8\ 786$ and $0\ 01 > p > 0\ 001$) The same was true for respiratory infections of more than 1 week duration ($\chi = 5\ 254$ and $0\ 02 > p > 0\ 01$)

Severity of illness as judged by the type of treatment the child received (outpatient or hospitalized) was greater in the Failing to Thrive Group 12 children from this group had been hospitalized at some time whereas

Table 1 *Education of parents*

	Thriving (48)		Failing to Thrive (57)	
	Fathers ( )	Mothers ( )	Fathers ( )	Mothers ( )
Koranic*	62.3	32.1	54.8	1.6
Primary	11.3	11.3	4.8	6.5
Secondary	9.4	0	1.7	0
None	17.0	56.6	38.7	91.9

* Able to read and write classical arabic

the mean age was 17.3 months while the Failing to Thrive Group had a mean age of 20.2 months This difference was not significant

(iv) *Sex of children* There were 24 boys and 29 girls among the Thriving and in the Failing to Thrive Group there were 25 boys and 37 girls

(v) *Birth size* Birth weights were unfortunately not available but estimates given by the mothers showed a higher proportion of lighter babies in the Failing to Thrive Group than in the Thriving Group

(vi) *Birth rank* The mean birth rank of the Thriving Group was 5.5 and is not significantly different from that of the Failing to Thrive (mean 6.1)

(vii) *Family size* The average number of children in the Thriving Group families was 4.4 (range 2–14) and in Failing to Thrive families was 5.1 (range 2–10) Large families (more than 4 children) were more common in the Failing to Thrive Group ($\chi^2 = 7.595$ and $0.01 > p > 0.001$)

(viii) *Siblings Index of Thriving* The Index of Thriving in the siblings of the Thriving Group (mean 3.6) was significantly lower than that of the Failing to Thrive Group (mean 5.3) ($0.02 > p > 0.01$) indicating better growth in the former

(ix) *Abortions and sibling deaths* The proportion of the mothers who had had an abortion was greater in the Failing to Thrive (44.6*) than in the Thriving (41.6 •) However abortions were more frequent in the Thriving than in the Failing to Thrive Group (18.5 • vs 10.8% of pregnancies $\chi = 7.640$ and $0.01 > p > 0.001$) The greater number of abortions in the Thriving Group may have been due to induced rather than to spontaneous abortions There were not significantly more deaths (10.3° of pregnancies) among siblings of the Failing to Thrive Group than in the Thriving Group (7.5)

Table 2 *Distribution of radios television sets and refrigerators*

	Thriving (48)		Failing to Thrive (57)	
	No		No	
Radio	44	87.3	44	78.7
TV	12	24.1	9	16.1
Refrigerators	35	65.8	8	31.1
Refrigerator + Radio or TV	33	63.3	15	27.9
None	1	6.3	8	14.5

2) *Socio-economic Data*

(i) *Education of parents* At all levels a greater proportion of both sexes in the Thriving Group was educated and in both groups fathers were better educated than mothers (Table 1) A higher percentage of fathers ($\chi = 6.62$ and $0.01 > p > 0.001$) and mothers ($\chi = 18.99$ and $p < 0.001$) in the Failing to Thrive Group had no education

(ii) *Occupation of parents* Most of the fathers were employed on the land or as casual semi skilled or unskilled workers in various trades (81.1 in the Thriving Group and 80.2% in the Failing to Thrive Group) In both groups few mothers admitted to working for people other than their families

(iii) *Income* The mean monthly income per family was significantly higher in the Thriving Group (£ L 242) than in the Failing to Thrive (£ L 213) $p < 0.001$ One Lebanese pound is about 30 U S cents There were significantly more families with an income in excess of £ L 250 in the Thriving Group ($\chi = 26.85$ $p < 0.001$) On a per capita basis the difference would be even greater No differences were observed within the same group in the three areas

(iv) *Household possessions* The greatest difference between the groups was in the possession of a refrigerator (χ = 17 91 and $p <$ 0 001) (Table 2) Within the same group the families in Jba a owned fewer refrigerators than families in the other two areas This may be due to the more conservative attitude of rural communities

3) *Living conditions*

Most of the houses are of concrete construction In both groups more than 60 of the houses were rented In Jba a however a higher proportion (75) of both groups owned their own houses than in the urban areas

(i) The Thriving Group lived in clean and well kept houses with more rooms than the other group For homes with 3 rooms and more they had a significantly higher percentage (χ = 16 19 and $p <$ 0 001)

(ii) Toilet facilities were usually inside the home in the Thriving Group (95 8*) and less often so in the Failing to Thrive Group (73 7*) (χ = 9 532 and 0 01 $> p >$ 0 001)

(iii) A special bathroom was more often found in the Thriving Group (85 4) than in the Failing to Thrive (52 6) (χ = 12 931 and $p <$ 0 001)

(iv) Water was available in the homes of the Thriving Group (95 8) significantly more often than in those of the Failing to Thrive (75 4) (χ = 8 35 and 0 01 $> p >$ 0 001)

(v) The kitchen was a special room in 97 9 of the Thriving Group while for 16 of the Failing to Thrive the food was cooked either outside or in one of the living rooms (χ = 5 77 and 0 01 $< p <$ 0 02)

Rural homes of both groups were usually larger than urban and general living conditions better

4) *Health Data*

(i) *Family health history* Cardio vascular system disease diabetes and cancer were all more common in the adults of the Thriving Group than in the Failing to Thrive Group though not significantly so

Table 3 *Mean daily calorie and protein intake*

	Thriving (.6) calories	protein	Failing to Thrive (45) calories	protein
No of cases	kcals	g	kcals	g
Mean intake day	543	1 8	389	17 9
Range	(360–840	(10 4–39 0)	(16..790)	(7 9–39 0)
of requirements Age 0–3 months				
above 85	14	25	12	39
below 85	12	1	33	6
of requirements Age 4–6 months				
above 85	6	4	3	31
below 85	.0	2	42	14

(ii) *Mother s antenatal history* Health during pregnancy did not differ significantly in the mothers of the two groups Morning sickness however was nearly twice as frequent in the Failing to Thrive Group

(iii) *Study child s medical history* Children in the Failing to Thrive Group had a higher prevalence of measles (29 0 v 26 4) and gastro-enteritis (80 7 v 67 9 %) but only for pertussis (12 9 v 1 9) was the difference significant (χ = 4 96 and 0 02 $< p <$ 0 05) Upper and lower respiratory infections were more common in the Thriving Group (75 5 v 61 3)

In both groups children experienced equally repeated episodes of both gastro-enteritis and upper and lower respiratory infections The estimated duration of illness tended to be shorter in the Thriving Group More of those who had suffered from gastro enteritis for more than 1 month were in the Failing to Thrive Group (χ = 8 786 and 0 01 $> p >$ 0 001) The same was true for respiratory infections of more than 1 week duration (χ = 5 254 and 0 02 $> p >$ 0 01)

Severity of illness as judged by the type of treatment the child received (outpatient or hospitalized) was greater in the Failing to Thrive Group 12 children from this group had been hospitalized at some time whereas

Table 4 *Ranking of factors significantly different in the two groups by χ^2 test*

	χ^2	p
1 Introduction of supplementary solid food before 6 months of life	26 278	$p<0.001$
2 Income more than 250 Lebanese pounds	20 541	$p<0.001$
3 Mother's education (able to read and write)	18 990	$p<0.001$
4 Refrigerator	17 910	$p<0.001$
5 House size (more than 3 rooms)	16 190	$p<0.001$
6 No vaccination	14 290	$p<0.001$
7 Indoor bathroom	12 931	$p<0.001$
8 Not hospitalized for a severe illness	12 350	$p<0.001$
9 Indoor toilet	9 532	$0.01>p>0.001$
10 Weaned during 1st and 2nd months	9 347	$0.01>p>0.001$
11 Gastro-enteritis for a period of more than 1 month	8 786	$0.01>p>0.001$
12 Water supply within the house	8 350	$0.01>p>0.001$
13 Family size (more than 4 children)	7 595	$0.01>p>0.001$
14 Bottle introduced during the first month	7 577	$0.01>p>0.001$
15 Father's education (able to read and write)	7 407	$0.01>p>0.001$
16 Caloric intake (less than 85 requirement)	6 076	$0.02>p>0.01$
17 Indoor kitchen	5 770	$0.02>p>0.01$
18 Respiratory infection for more than 1 week	5 254	$0.05>p>0.02$
19 Protein intake (less than 85 of daily requirement) for the age period 4–6 months	5 082	$0.05>p>0.02$
20 Pertussis	4 960	$0.05>p>0.02$

only one of the Thriving Group had been in hospital ($\chi^2=12.35$ and $p<0.001$)

The educational levels and occupation of the parents had no significant influence on the frequency of infection of the children in the study

(iv) *Immunization* Although significantly more children (75%) in the Thriving Group had received immunization, than in the other group (40%) ($\chi^2=14.29$ and $p<0.001$) few mothers in either group were following proper immunization schedules

5) *Dietary Data*

(i) *Bottle feeding* Among the 40 Failing to Thrive and 26 Thriving bottle fed children a significantly greater number in the Failing t[o] Thrive group were bottle fed in the first months (57 5 % vs 38 5% $\chi^2=7.577$ an[d] $0.01>p>0.001$)

In the Thriving Group 84 6% and in th[e] Failing to Thrive Group 82 5% gave insuff[i]cient or no milk as the reason for bottle fee[d]ing A further 7 7% Thriving and 10 0% Fai[l]ing to Thrive gave a subsequent pregnancy [as] the reason 81 1% of Thriving children ha[d] been breast fed for at least 6 months, or wer[e] still being breast fed but only 55 6% of th[e] Failing to Thrive Group ($\chi^2=16.177$ and $p<$ 0 001)

(ii) *Weaning* Reasons for weaning wer[e] given as insufficient milk (42 3% Thrivin[g] and 55% Failing to Thrive) and subsequen[t] pregnancy (38 5% and 27 5 % respectively) There was a significantly higher percentage o[f] children of the Failing to Thrive group weane[d] before the fourth month (37 1% v 15 1% $\chi^2=7.057$ and $0.01>p>0.001$)

(iii) *Introduction of solid food* By the ag[e] of 6 months 75 5% of the Thriving Grou[p] had received solid food compared with 27 4% of the Failing to Thrive Group ($\chi^2=26.278$ an[d] $p<0.001$) This usually consisted of family diet and specially prepared food such a[s] mashed fruit porridge eggs and vegetabl[e] soup

(iv) *Estimated energy and protein intakes* These are calculated for bottle fed babies from information on concentration of the formula volume and frequency given by the mother

Calorie and protein intakes per child pe[r] day were significantly higher in the Thriving Group than in the Failing to Thrive ($t=$ 4 053 and $p<0.001$ and $t=2.191$ and $0.05>p>0.02$ respectively) (Table 3) A greater per centage of children who received below 85 % of their requirements of calories were in the Failing to Thrive group than in the Thriving Group ($\chi^2=6.076$ and $0.02>p>0.01$ for the age period 0–3 months and $\chi^2=4.016$ and $0.05>p>0.02$ for the age period 4–6 months) There was no significant difference between the groups with regard to protein intake dur

ing the first 3 months but the protein intake was significantly higher in the Thriving Group for the next 3 months (χ = 5 082 and 0 05 > p > 0 02)

DISCUSSION

When those factors which differ significantly between the two groups are ranked according to the χ value of the difference the multi factorial nature of failure to thrive is evident Comparison of the three communities showed that for both groups Jba a families are consistently the best and Basta the worst and that any Thriving Group was better than the best Failing to Thrive group (from Jba a)

Dietary factors adversely affecting the development of the children were most commonly inadequate caloric intake bottle feeding and early weaning These factors are most obvious when results from Jba a are compared with those from the urban areas

The general health history of the family appeared to have little influence on the Thriving Index of the child but the medical history of the child itself was an influence with severity of infection and the duration of the illness being more important than its type The difference in the Index of Thriving of siblings of the study children in the two groups suggests that the various factors influence all young children in the families in the same way if not to the same extent

In a similar study to ours in rural Mexico (2) on 26 children who gained less than 510 g per month during the first 6 months of life and 26 who gained over 750 g the factors most associated with poor growth during these month were cultural and social diet appeared to be a minor factor However the difference in growth of these two groups was considerably less than in ours It is known from another study that the money spent on food in the families of both groups is similar (7) and it seems probable that the difference in energy intake of the children is due more to the way in which the food is being shared among different members of the family than to its availability alone That the major deficit is in energy rather than protein is consistent with other studies (9 10 11)

The general conclusion from this study is that in low socio-economic circumstances factors other than food and health are involved in adequate growth of the pre school child Living conditions and maternal care appear to be major factors Many of the apparent causes of failure to thrive are directly related to income and form a chain of interrelated factors A family which can only afford a low grade house without water supply is automatically barred from its own toilet and bathroom facilities and the possession of an inside kitchen is made more difficult Expensive items such as refrigerators and television sets are more available to families where there is just a little to spare for luxuries The cost of medical advice in such families results in delay of proper treatment of childhood diseases which in turn can result in the illness being allowed to develop in severity The difference in the mean income of the two groups is only £ L 30 ($10) per family per month

SUMMARY

From among more than 1 200 children aged 3–48 months of low socio-economic families living in rural suburban and urban Lebanon two contrasting groups were selected according to their Index of Thriving for detailed study at home (53 Thriving and 62 Failing to Thrive) Family socio economic living conditions health and dietary data were obtained Numerous statistically significant differences were found in all categories of data between the two groups with poorer status invariably shown by the Failing to Thrive families The implications of the multi factorial nature of the causation of failure to thrive are discussed

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RENAL FUNCTION IN LONG TERM SURVIVORS AFTER TREATMENT FOR NEPHROBLASTOMA

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The problem presented by dysfunction of the remaining kidney after successful treatment for nephroblastoma may be expected to assume increasing importance in view of the greater proportion of survivors the higher doses of radiation and the use of supplementary Actinomycin D

It has been observed that doses in excess of about 2 300 rad delivered to the whole of both adult kidneys can result in impaired renal function (16) It has been suggested that renal function compatible with clinical health in nephrectomized children can be preserved as long as the remaining kidney has received less than about 1 200 rad (20) Fatal renal failure after 18 symptom free years has been reported in a patient given about 1 400 rad to the remaining kidney at the age of 3 months (19)

The underlying process of late radiation damage is progressive nephrosclerosis In the pathogenesis of radiation induced nephrosclerosis the arterioles and capillaries are of primary importance The renal epithelium seems to be relatively resistant to the direct action of radiation but may degenerate as a result of damage to the fine vasculature The proximal tubuli are apparently more affected by radiation than the distal ones (6 18 22)

When renal tissue has been removed or damaged the remaining nephrons increase in size and function (14) After nephrectomy the filtration capacity of the glomeruli increased to 70–85 of the previous combined capacity of the intact kidneys The renal tubuli undergo hypertrophy and increase their reabsorption (12)

Pertinent questions in this respect are (*a*) whether there is evidence of renal dysfunction in long term survivors after irradiation for nephroblastoma and (*b*) whether the degree of dysfunction if any is related to the radiation dose received by the remaining kidney during the primary treatment (*c*) whether the degree of dysfunction if any is related to the time interval after radiation The aim of this investigation was to find out if relevant information concerning these points could be achieved in the available material of nephroblastoma patients Those aspects of renal function have been investigated which have been considered to be most important for the homeostatic regulation

MATERIAL

Between 1952 and 1970 a total of 63 patients with localized unilateral nephroblastoma were treated at Radiumhemmet 34 of these patients died in nephroblastoma without evidence of renal failure and 3 had metastases at the time of this study Of the 26 patients alive without sign of the disease at least 2 years after the last treatment 4 were less than 6 years old and too young to be able to co-operate The parents of 6 refused to allow the patient to be admitted to hospital

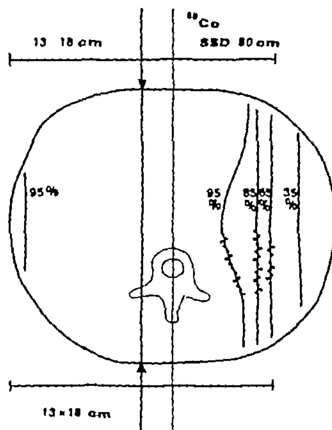


Fig 1 Reconstruction of dose distribution in one of the patients treated by cobolt 60 unit

Sixteen patients remained available for examination of renal function At the time of the primary treatment they were between 1 and 6 years old The time elapsing between the primary treatment and the study ranged from 2 to 19 years

Of these 16 patients one had undergone total nephrectomy only the other 15 nephrectomy and postoperative irradiation In all but one unilateral nephrectomy was complete

All patients were followed up regularly One was treated for pulmonary metastases 3 years after the primary treatment and at the time of the study had been 4 years without evidence of the disease Fifteen had had no evidence of disease since the time of the primary treatment None of the 26 patients showed any clinical sign of renal dysfunction during the follow up period

To examine whether the radiation dose had any bearing on the results the patients were divided into two groups one (group A) consisted of 7 patients treated with a conventional X ray unit in whom the mean dose to the remaining kidney was between 250 and 650 rads in 14–42 days and one patient not receiving irradiation The other (group B) comprised 8 patients treated with a cobolt 60 unit in whom the mean dose to the remaining kidney was between 1 200 and 1 700 rads in 34–63 days There were no patients treated with doses 650–1 200 rads to the remaining kidney

METHODS

Seven patients were treated with a conventional X ray unit (170 kV 0 5 mm Cu SSD 50 cm) and 8 with a cobolt 60 unit (SSD 70 cm) The treatment technique was similar in all patients namely 2 large opposing fields covering the area of the operation from the xyphoid to either symphysis or iliac crest In 4 patients given X ray therapy the medial limit of the field was located in the midline In the remaining 3 and in the patients given treatment by the cobolt-60 unit the medial limit was located beyond the midline In 5 of the patients treated with a cobolt-60 unit the remaining kidney was shielded by a piece of lead 5 cm thick after delivery of a tissue dose of 1 500 rad in the centre of the beam The positions of the fields and the lead were checked radiographically

In 11 of the 15 patients receiving radiotherapy Actinomycin D was added at the initial treatment In 9 patients one course of Actinomycin D was given at the time of the operation and 2 patients received several courses of Actinomycin D over a period of 15 months after the operation The total dose of Actinomycin D per course was 60 gamma/kg body weight applied during 6–8 days

The dose distribution in the remaining kidney was reconstructed on the basis of renal radiographs obtained at the time of primary treatment and the relevant information in the treatment charts namely exposure dose field size and position source skin distance diameter of the patient and measurements of the entrance and exit doses (Fig 1) The maximum minimum and mean doses to the remaining kidney in relation to the maximum tissue dose in the tumour area and to the treatment time are shown in Table 1

In the patients treated with the conventional X ray unit the remaining kidney was exposed to scattered radiation only The estimated error was ±20% of the calculated dose for patients treated with cobolt 60 and 25 for the patients treated with conventional X ray irradiation

In all the patients renography was performed twice namely at the time of this study and one year previously The equipment consisted of two sodium iodine detectors (crystal size 1 3/4 ×1") connected to a digital ratemeter (Picker Dual Rate Computer) this integrated the pulses from the detectors over an interval of 6 seconds and after each interval the values were registered by means of a two-channel recorder The counting time was 20 minutes The amount of injected activity was 0 6 μCi 131I labelled Hippuran/kg body weight

The other renal function tests were performed at the paediatric metabolic ward of St Goran s Hospital Stockholm The patients were hospitalized for 5 days Urine cultures and analyses were carried out during the first day in hospital Blood samples were taken for determinations of urea electrolytes ESR Haemoglobin and haematocrit The blood pressure was measured with a cuff The tests were performed during water diuresis (3) The following aspects of the renal function were examined during the next 4 days

Table 1 *Radiation dose and time at primary treatment and follow up period*

Patients	Radiation dose rad				Radiation time (days)	Follow up period (year)
	To the tumour bed	To the remaining kidney				
		Max.	Min	Mean		
Group A						
B O	1 200	300	100	250	17	19
A.	1 200	300	100	250	14	18
S	2 900	600	200	500	35	14
L A	3 750	800	300	650	35	11
B P	2 400	500	200	400	21	11
E	2 000	500	200	400	31	8
A K.	2 800	600	200	500	42	6
B J	—	—	—	—	—	6
Group B						
K. E.	2 700	2 400	600	1 600	63	6
E. W	2 900	2 800	400	1 700	39	5
P L.	3 000	2 800	200	1 600	40	5
M W	3 000	2 700	200	1 200	34	5
H O	3 000	1 600	1 100	1 450	39	4
B Z.	3 000	1 600	1 200	1 500	45	2
M P	3 100	1 500	1 200	1 400	49	2
R. K	3 000	1 400	900	1 300	47	2

1 Glomerular filtration rate and PAH clearance (day 2)

2. Renal regulation of sodium balance (day 2)

3 Renal regulation of acid base balance (day 4)

The glomerular filtration rate and the clearance of PAH were determined by single injection technique (8) During water diuresis and before sodium chloride was administered a single injection of a solution containing 9% of inulin (Laevosan Gesellschaft) and 10% of para aminohippuric acid (PAH) 0 075 ml/kg body weight was given intravenously Blood samples were taken at 5 minute intervals during the first 70 minutes after the injection and at 10-minute intervals during the next 60 minutes From the plasma disappearance rate thus obtained the clearance of inulin (C_{in}) and PAH (C_{PAH}) was calculated from the formula given by Sapirstein (23) The filtration fraction (FF) was calculated as the quotient of the glomerular filtration rate (clearance of inulin) by the clearance of PAH The concentration of inulin in the blood was determined by the Antrone method (13) and the concentration of PAH by the method of Smith (24)

To examine the renal regulation of the sodium balance an oral salt load was given This test had previously been carried out in 7 healthy children at the same laboratory (4) The urine samples were collected by spontaneous voiding by hourly intervals the first of them 2–3 hours after starting the high fluid intake During the second collection period the oral salt load was given as sodium chloride tablets (ACO) 95 mEq/1 73 m BSA these were ingested during the first 15 minutes of the second urine collection period After the administration of sodium chloride 5 or 6 urine samples were collected and the sodium concentration was determined by a flame photometer

The regulation of the acid base balance was tested by examining the effects of an oral load of ammonium chloride (9) After sampling of blood and urine ammonium chloride 150 mEq/m² b s was given orally Five urine samples were then collected and blood samples were taken 2 3 and 5 hours later The pH in blood was determined with a pH meter (radiometer) Standard bicarbonate was determined by analysing the pH after equilibrating blood samples with 4 and 8 % of carbon dioxide By plotting the pH results on a Siggaard Andersen curve nomogram the P_{CO_2} standard bicarbonate and total CO_2 concentration were obtained (21) The results of the ammonium chloride test were interpreted from the relationship between the urine pH and the concentration of total blood CO_2 In healthy children this relationship has been found to be characteristic (3)

Values for GFR C_{PAH} and FF lying in the range mean ± 1 S D for two kidneys in healthy children were regarded as normal

RESULTS

In all the children the haemoglobin erythrocyte count white blood cell count thrombocyte count haematocrit the electrolyte level

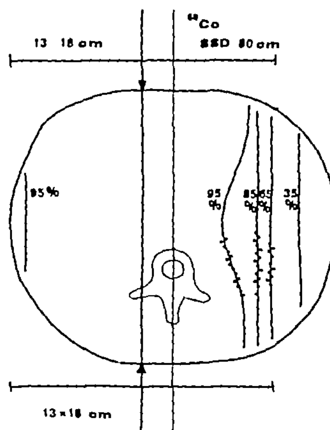


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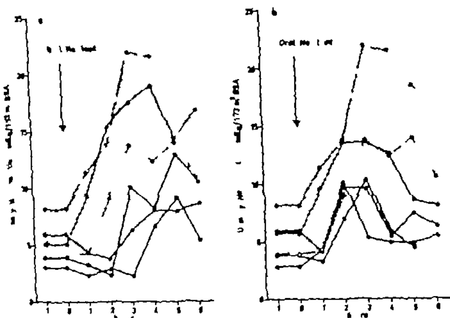


Fig Hourly sodium excretion following an oral sodium load of 95 mEq/1 73 m BSA (*a*) in patients given 250–650 rad/17–42 days and (*b*) in patients given 1 200–1 700 rad/34–63 days The shaded field indicates means for healthy children ±1 SD (4)

sodium excretion greater than $x+1$ SD for normal children on well standardized diet and were therefore excluded from further study[1] In one patient the test failed The hourly sodium excretion in relation to then in normal children is shown in Figs 2*a* and 2*b* ($x \pm 1$ SD for healthy children indicated in the figures) In one of the 4 patients in group A (A K) the hourly excretion was within the normal range and in the remaining 3 patients it was decreased (H A G P and J A) (Fig 2*a*) The excretion curves were similar to those in normal children in the first 2 hours there was an increase in the excretion following the sodium load and during the next 4 hours the level was fairly stable (1) Of 4 patients from group B one (E W) showed a normal response while in the other 3 it was decreased (M P M W and H O) (Fig 2*b*)

The renal regulation of acid base balance was examined in 13 children in 3 of whom it failed technically The results are presented in Figs 3*a* and 3*b* Of the 6 patients of group A in whom the regulation of the acid base balance was examined the results were interpreted as normal in 2 (G P and J A) borderline in 2 (B S and B J) and pathological in 2 (H A and A K) In the 2 patients with a pathologically low urine acidifying capacity the blood total CO concentration had to be depressed to 15 mM/l (standard bicarbonate about 14) before the urine pH fell In all 6 patients however the urine pH fell below 5 5 when the total CO in blood had been depressed enough (Fig 3*b*)

In the group B patients the results were interpreted as normal in 2 (E W and M P) and as pathological in 5 (H O R K P L K E and B Z) In these 5 patients the urine pH remained above 5 5 even when the blood CO concentration was as low as 15 mM/l (Fig 3*b*)

[1] For technical reasons the children of the present study could not be kept on standardized diet prior to the study The reasons for the high basal salt excretion might have been dietary variations

Table 2 Glomerular filtration rate (GFR) clearence of PAH (C_{PAH}) and filtration fraction (FF) in 16 nephroblastoma patients surviving 2–19 years after treatment

Patient	Age at treatment	Age at study	GFR (ml/min/1 73 m²)	C_{PAH} (ml/min/1 73 m²)	FF ( )	Blood pressure (mm Hg)
Group A						
B O	4	23	52 2	376	14	125/80
J A	5	23	133 0	581	23	130/80
B S	1	15	113 0	456	25	110/75
H A	2	13	96 0	540	18	110/75
G P	3	14	86 0	487	18	110/70
P E	2	10	92 0	422	22	120/70
A K	3/12	6	123 0	549	22	90/50
B J	2	8	96 0	415	23	120/70
Mean ± 1 S D			98 9±24 9	478±73 3		
Group B						
K E	4	8	115 0	397	29	120/70
E W	5	10	118 0	579	20	125/80
P L	6	11	101 0	494	20 5	120 80
M W	4	9	88 0	—	—	100/65
H O	5	7	94 0	481	20	120/75
B Z	6	8	108 0	426	25	100/70
M P	5	7	104 0	418	23	110/70
R K	4	7	112 0	487	23	110/70
Mean ± 1 S D			105 0±10 6	469±61 7		
Mean ± 1 S D for 7 healthy children aged between 8 and 14 years			122 5±16 4	546 0±47 2	21 2±1 3	
Mean ± 1 S D for 29 healthy adult kidney donors (15)			91±17 2	368±98		

and urea concentration in the serum and the sedimentation rate were within the normal range and likewise the blood pressure lay within the range for normal children of the same age (Table 2) (11) None of the children had clinical or bacteriological evidence of urinary tract infection at the time of the investigation In none of the children did renography reveal impaired renal function

The values of the glomerular filtration rate (GFR) PAH clearance (C_{PAH}) and filtration fraction (FF) are presented in Table 2

In 7 out of the 16 children in whom the GFR was examined the values were within normal limits In 6 they were less than $\bar{x}-1$ SD 3 of them from group A (H A P E and B J) and 3 from group B (P L H O and M P) in 3 the values were lower than $\bar{x}-2$ SD 2 of them from group A (G P and B O) and one from group B (M W) In all but one of the patients the GFR was more than 50 of the normal value for healthy children with both kidneys The value was within the normal range for *one* kidney in the remaining one patient (B O)

The C_{PAH} and FF were examined in 15 children In 4 of these the C_{PAH} was within normal limits In 4 it was less than $\bar{x}-1$ S D 2 of them from group A (B S and G P) and two from group B (H O and R K) In 7 the value was lower than $\bar{x}-2$ S D 3 of them from group A (B O P E and B J) and 4 from group B (K E P L B Z and M P) The C_{PAH} always exceeded the normal value for *one* kidney for healthy children with both kidneys intact The percentage of FF was within normal limits in 9 out of the 15 patients in 3 patients (B O H A and G P) from group A it was less than $\bar{x}-2$ S D In another 3 patients the percentage of FF was greater than $\bar{x}+2$ S D one patient (B S) from group A and 2 from group B (K E and B Z)

The renal sodium balance was examined in 8 patients Seven of the 16 patients had a basal

values less than the equivalent of 93 ml/min/1.73 m² have been found in 27 of the children examined 6 months to 18 years after treatment. Low values were more frequent in children who received more than 2 400 rads than in those receiving lower doses than this to the remaining kidney. The values for the glomerular filtration rate in our patients measured as the clearance of inulin are similar. They exceed the normal range for one kidney but there was no correlation with the radiation dose (Fig. 4). The renal regulation of the sodium balance and of the acid base balance was not investigated in that study (20).

In the present study the effective renal plasma flow as measured by the clearance of PAH was increased in proportion to the GFR and in most of the patients the FF values were thus normal. The abnormal FF values found in the 6 patients display neither a uniform pattern nor any correlation to the radiation dose. The C_I and C_{PAH} values indicate an increase in the surface of the glomerular capillaries and in the total blood supply in the remaining kidney. They are consistent with the expected hypertrophy and increase in function as compensation after nephrectomy (15). There was only one patient in the series (B. O.) in whom the GFR values did not suggest hyperfunction. This patient has the longest observation time but received a low dose of radiation which might be an indication that the time lapse might be of importance for renal dysfunction (Fig. 4).

The renal response to the ammonium chloride load was pathological in several patients, more so in those who had received a higher radiation dose. In some of the group A patients the blood CO₂ level had to be lower than normal to depress the urine pH. This type of response suggests an inability to completely reabsorb bicarbonate (3, 9). In some of the group B patients there was an almost complete inability to depress the urine pH. This suggests an additional impairment of the excretion of the titratable acid and ammonia (3, 9).

The examination of the regulation of acid base balance in terms of the relation between the blood CO₂ and the urine pH is however only a *qualitative* test of tubular function. The fact that none of the patients had manifest or compensated blood acidosis suggests that only a small proportion of their nephrons were damaged with respect to bicarbonate secretion and that the major proportion of the nephrons remained intact to maintain the acid base balance under ordinary conditions.

The absence of an adequate response to the sodium load was the most significant result of this study. There was no evident correlation of the sodium excretion rate with either the GFR or the dose of radiation. Theoretically the hyperperfusion of the kidney might increase the sodium transport from the renal interstitium to the systemic circulation and thereby the reabsorption of sodium.

The lowered hourly sodium excretion observed in some of the patients might be of some clinical importance since a connection has been shown to exist between defective sodium homeostasis and future development of hypertension (7).

SUMMARY

Renal function was investigated in 16 cases of nephroblastoma in whom nephrectomy had been performed 2–19 years previously. All but one of these patients were also given postoperative radiation. The average radiation dose to the remaining kidney ranged from about 250 to about 1 700 rads in 17–63 days.

None of the patients had clinically manifest renal dysfunction. The glomerular filtration rate was examined in 16 patients, the clearance of PAH and the filtration fraction in 15, the renal sodium balance in 8 and the acid base balance in 13.

The glomerular filtration rate and the clearance of PAH were 70–100 per cent of the normal values for healthy children with 2 kidneys. Subclinical impairment of the sodium balance control was found in 6 patients and of the

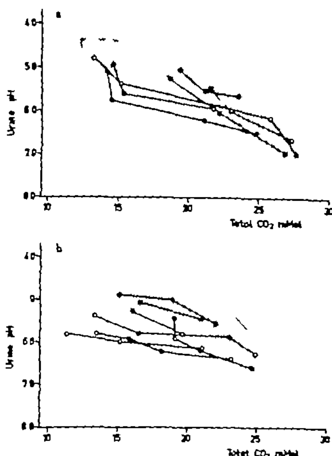


Fig 3 The relationship between total blood CO_2 and urine pH (*a*) in patients given 250–650 rad 17–42 days and (*b*) in patients given 1 200–1 700 rad/34–63 days The shaded field indicates the outer limits of values found in healthy children (3)

DISCUSSION

The evaluation of the results of this study might be questioned in several respects

1 The somewhat uncertain position of the reconstructed medial field limit might have been a source of error when calculating the radiation dose A movement of the midline for ±0 5 cm for instance would cause an error of ±20% in the calculated average dose to the remaining kidney The maximum dose however would remain rather constant

2 The patients have been divided into two groups as regards the dose of radiation they had received One group treated during the fifties and early sixties had received lower doses of radiation (group A) The other group of patients treated with higher radiation doses (group B) is from a later period The effect of radiation dose or the observation time on the results of investigation could therefore not be separated

3 A control group of children who were nephrectomized but did not receive radiation was not available There was only one such patient in our series (B J) The values obtained in our patients have been compared to the values obtained in healthy children with 2 kidneys (1 3 4 5)

Acute effects of radiation on the glomerular filtration rate and on the renal blood flow have been studied in experimental animals and man In dogs an initial increase in GFR and RPF was found during the course of radiation and some days afterwards followed after a few days by a permanent decrease the magnitude of which was dependent on the radiation dose (17) In man a reduction in the GFR as reflected in the clearance of creatinine has been observed in patients treated with radiation for the carcinoma of the uterine cervix (10) In another study of the acute effects of radiation on renal function in 10 patients with malignant tumours the most significant effects was on the renal plasma flow (RPF) which was reduced during the course of radiotherapy and was considered to be the most sensitive and consistent index of radiation damage (2)

The late effects of radiation on the renal function has been studied in 108 children after nephrectomy and postoperative radiation for malignant tumours (20) Creatinine clearance

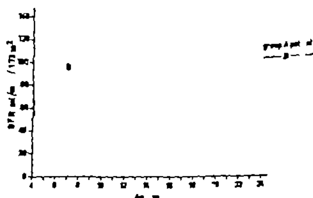


Fig 4 Glomerular filtration rate in relation to the age of the patient and radiation dose to the remaining kidney Mean ±1 S D for one healthy kidney marked with shaded field (5)

values less than the equivalent of 93 ml/min/1.73m have been found in 27% of the children examined 6 months to 18 years after treatment. Low values were more frequent in children who received more than 2 400 rads than in those receiving lower doses than this to the remaining kidney. The values for the glomerular filtration rate in our patients measured as the clearance of inulin are similar. They exceed the normal range for one kidney but there was no correlation with the radiation dose (Fig. 4). The renal regulation of the sodium balance and of the acid base balance was not investigated in that study (20).

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acid base balance in 9 Abnormal regulation of the acid base balance was more noticeable in the patients given higher radiation doses The glomerular filtration rate clearance of PAH, filtration fraction and the sensitivity of the sodium balance control were not correlated to the radiation dose

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RESPONSE OF THE JEJUNAL MUCOSA TO COW'S MILK IN THE MALABSORPTION SYNDROME WITH COW'S MILK INTOLERANCE

A Light and Electron Microscopic Study

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During the last few years attention has been paid to a clinical syndrome consisting of malabsorption, mucosal damage of the small intestine and intolerance to cow's milk (4, 7, 8, 10, 11, 21). This syndrome usually appears within 3 months after birth and disappears by about the age of 1 year. The intestinal mucosa displays various degrees of villous atrophy, the changes being sometimes similar to those found in coeliac disease (7, 21). Intolerance to cow's milk has been presumed to be an allergic reaction to cow's milk proteins (4, 5, 9). Whether cow's milk can directly produce a mucosal lesion is not known.

The present report concerns the light and electron microscopic findings in 3 infants suffering from this syndrome. Particular attention was paid to the effect on the small intestinal mucosa of alternating provocation with and elimination of cow's milk.

MATERIALS AND METHODS

Patients

This study was made in 3 consecutive cases. The infants fulfilled the following criteria: gastro-intestinal symptoms, malabsorption verified by absorption tests, disappearance of symptoms after elimination of cow's milk, and clinical reaction to cow's milk provocation.

The illness was manifested in all 3 patients at the ages of 0.5–2.0 months (Table 1). The symptoms in all were prolonged diarrhoea with vomiting and poor gain in weight (Fig. 1). Before admission, 2 of the 3 patients had been fed with cow's milk formula not containing wheat and one with formula that did contain wheat. In hospital, malabsorption (Table 2) and proximal jejunal villous atrophy of various degree were verified (Table 3).

Eliminations and provocations

When the patients were put on breast milk, the symptoms disappeared and the patient started to gain weight either directly or after some latent period (Fig. 1). When the clinical condition was satisfactory, a provocation test was performed with a cow's milk formula made from unadapted powdered cow's milk (100 ml of which contains 2.2 g proteins, 2.5 g fats, 0.26 g sucrose and 7.28 g lactose). Each provocation started with a single oral dose of 5 ml. If no clinical reaction was observed, the patients was given 10 ml with every meal and the dose was gradually increased every day. Within a week the patients were put on the cow's milk formula alone, if they had shown no reaction. The

Table 1. *Data on the patients' sex and their ages in relation to the medical history*

	Patient 1	Patient 2	Patient 3
Sex	Female	Male	Female
Age at first feed containing cow's milk (months)	0.5	0.7	0.5
Age at first feed containing wheat (months)	—	2.0	—
Age at onset of symptoms (months)	0.5–1.0	1.5–2.0	0.5–1.0
Age on admission (months)	1.6	3.3	3.5

Table 2 *Results of investigations on admission during treatment with breast milk and after provocation*

Patient	Age (months)	Time of investigation	Faecal fat excretion (g/day)	fat absorbed from diet	Urinary D-xylose excretion in 5 hours ( )	Serum IgA level (mg/100 ml)
	1 6	Admission start of elimination			5	36
1	3 0	1 4 months on elimination just before provocation	6 6	71		9
	3 0	20 hours after a single challenge with cow s milk				73
	6 0	Elimination 4 4 months	0 4	95		8
2	3 3	Admission start of elimination	13	48	4 ?	99
	6 0	2 1 months on elimination	3 2	88	15 3	20
	7 2	3 1 months on elimination just before provocation	3 6	72	15 4	0
	7 3	4th day on provocation				86
	8 5	38th day on provocation start of re elimination	6 3	84	1? 1	54
	9 5	Elimination 30 days				34
3	3 5	On admission	5 4	77	14 7	84
	4 2	Elimination 6 days				56
	4 8	Elimination 33 days start of provocation	2 9	89	20 9	22
	6 2	Duration of provocation 41 days	4 6	83	8 3	135

cow s milk diet was continued until symptoms appeared

When provoked with a single dose of 5 ml cow s milk formula at the age of 3 0 months patient 1 reacted in 2 hours with vomiting mucus containing stools shock like pulse and sweating The provocation test was discontinued A similar rapid but milder reaction was provoked again at the age of 4 4 months The third challenge test at the age of 6 months resulted in anorexia and loss of weight after 10–14 days which we call the slow reaction The challenge tests at the age of 4 4 months and 6 months respectively were performed only to find out if the patients could already tolerate cow s milk and if they could be discharged on a diet containing cow s milk No biopsies were done in connection with these two challenge tests This study concerns only the first provocation When patient 2 was provoked with cow s milk at the age of 7 2 months the intolerance manifested itself by degrees as anorexia tiredness and failure to thrive in 20 days (Fig 1) after the commencement of milk feeding After 38 days challenge the gain in weight had stopped and the challenge had to be discontinued At that time the D-xylose excretion decreased The faecal fat excretion and the serum IgA level increased (Table 2) The clinical intolerance to cow s milk disappeared at the age of 16 months Provocation produced the same type of reaction in patient 3 as in patient 2 but the symptoms were milder and did not appear until 35–40 days after the start of the provocation (Fig 1) Simultaneously faecal fat excretion and the serum IgA level increased perceptibly and the D xylose excretion decreased (Table 2) After the symptoms appeared cow s milk was again eliminated At the age of 1 3 years patient 3 could tolerate cow s milk well

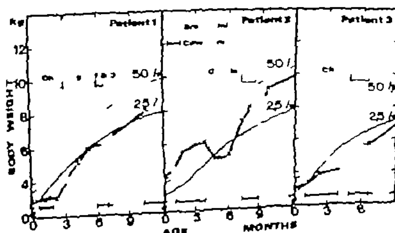


Fig 1 The weight curves of the patients on the elimination diet and during provocations with cow s milk The percentiles 50 and 25 indicate the normal weight curves for Finnish children

Precipitins to	
cow's milk	gluten
Negative	Negative
Negative	Negative
Negative	Negative
Positive (weak)	Negative
Positive (weak)	Negative
Negative	Negative
Negative	Negative
Positive	Negative
Positive (weak)	Negative
Negative	Negative
Positive (weak)	Positive (weak)
Positive (weak)	Negative
Positive	Negative
Positive	Negative

Methods

Biopsies were done before and during provocations as indicated in Table 3. Specimens were taken from the proximal jejunum near the ligamentum of Treitz with a Crosby Kugler capsule of paediatric size. Each specimen was immediately immersed in 2 cacodylate buffered glutaraldehyde (pH 7.2) and studied under a dissecting microscope (50). The specimen was subsequently cut in two. One part was fixed in phosphate buffered 4% formaldehyde (pH 7.4) for routine histological examination and the other was kept in the glutaraldehyde solution for 6 to 12 hours. Paraffin sections were stained with haematoxylin eosin (HE) and PAS stain. The height of the surface epithelial cell is expressed as the median height of 50 epithelial cells chosen at random. The intra-epithelial round cell count was evaluated by counting the number of intra epithelial round cells per 500 epithelial cells. The result was expressed as a percentage. The biopsy specimen for electron microscopy was rinsed several times in cacodylate buffer and postfixed in 1% phosphate buffered OsO dehydrated in graded alcohols and embedded in Epon 812 resin. Ultrathin sections were stained with uranyl acetate and Pb citrate and studied with a Zeiss EM 9 A electron microscope. Details of the evaluation of the jejunal biopsy specimens (3, 7, 24), performance of IgA measurements (6, 12) and the absorption tests have been described in earlier reports (7, 24).

Consent to the procedure, which was described as being exclusively for diagnostic and research purposes and thus devoid of any therapeutic or palliative effect, was obtained from the parents.



Fig. 2. Jejunal mucosa of patient 3 before any treatment. It shows subtotal villous atrophy with a decreased height of surface epithelium. HE × 120.

Table 2 *Results of investigations on admission during treatment with breast milk and after provocation*

Patient	Age (months)	Time of investigation	Faecal fat excretion (g/day)	fat absorbed from diet	Urinary D-xylose excretion in 5 hours ( )	Serum IgA level (mg/100 ml)
	1 6	Admission start of elimination			25	56
1	3 0	1 4 months on elimination just before provocation	6 6	71		9
	3 0	20 hours after a single challenge with cow s milk				7 5
	6 0	Elimination 4 4 months	0 4	98		8
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3	3 5	On admission	5 4	77	14 7	84
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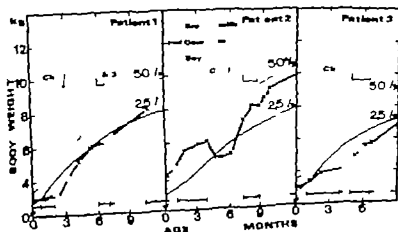


Fig 1 The weight curves of the patients on the elimination diet and during provocations with cow s milk The percentiles 50 and 2 5 indicate the normal weight curves for Finnish children

lectron microscope		
hort bnormal icrovilli	Nuclei of abnormal shape and position	Thickening of basement membrane
.	+	–
.	+ +	+ +
	–	–
.	–	+
lot done	Not done	Not done
+	+ +	+ +
	+	+
· +	+	+
	–	–
·	–	–

casional nuclei were amoeba like The epithelial basement lamina was unevenly thickened but without collagen fibres or other abnormal deposits (Fig 4)

Provocation with cow's milk

Patient 1 reacted rapidly The changes could already be seen under a light microscope in a biopsy specimen taken 20 hours after a single challenge with cow's milk The most striking change was the "round cell infiltration into the surface epithelium and the lamina propria The height of the surface epithelial cells had also decreased (Table 3) Electron micrographs of this biopsy specimen showed short normality of nuclear shape and loss of perpendicular orientation of the nuclei and infiltration of lymphocytes into the surface epithelium Some increase in the number of lysosomes was noticed in the apical part of the cells

In the slow reacting patient No 2 similar cellular infiltration and epithelial changes were found under a light microscope in a biopsy specimen taken 4 days after the start of provocation (Fig 5 before provocation and Fig 6 after provocation) At this time the patient had no symptoms Later (38 days after the start of the provocation) the patient already had symptoms and the changes as mentioned above were much more in evidence (Fig 1 and Table 2) In electron microscopy the microvilli were short and frequently fused at their bases Lysosomes had accumulated in the apical part of the cells (Fig 7) and the nuclei had lost their perpendicular orientation Furthermore this biopsy showed continuous accumulations of undulating and whirled collagen fibres at the thickened basal lamina (Fig 8) This feature was not noticed in specimens from either of the other patients taken at various phases of the disease

The reaction after 41 days of cow's milk challenge in patient 3 was much less pronounced than in patient 2 She showed round cell infiltration into the surface epithelium and the lamina propria but no decrease in the height of the surface epithelial cells In the electron microscope we could see some shortening of the microvilli

The effect of elimination

The withdrawal of cow's milk from the diet for about a month in patients 2 and 3 (from the initial situation in the latter) normalized the structure of the surface epithelium considerably in both light and electron microscopy (Table 3) The biopsy from patient 1 after a diet containing no cow's milk for 4 5 months displayed an almost normal epithelium in light and electron microscopy Some empty looking areas were encountered possibly due to a fixation artefact of the absorbing cells (Fig 9)

Table 3 *Changes in the small intestinal mucosa visible in dissecting light and electron microscopy*

Time of biopsy	Dissecting microscope appearance of the small intestinal mucosa	Light microscope: Villous[a] architecture	Epithelial injury	Median height of epithelial cells (μm)	Intra epithelial round cell infiltration ( )
Patient 1					
After 1 3 months on elimination, just before provocation	High and low ridges	PVA	+	27 9	7 4
20 hours after a single challenge dose of cow s milk	Low ridges	PVA	+ +	22 5	14 4
Elimination 4 5 months	Finger and leaf like villi	Slight mucosal changes	–	32 9	4 4
Patient 2					
After 3 1 months on elimination just before provocation	Low ridges	SVA	+ or ±	27 0	4 8
4th day of provocation	Low ridges	SVA	+ +	23 4	8 4
38 days of provocation	Flat mucosa with mosaic pattern	PVA	+ +	21 6	15 2
Duration of re-elimination 30 days	Low ridges	SVA	+	31 3	7 0
Patient 3					
Admission duration of elimination 0 days	Flat mucosa with mosaic pattern and some low ridges	SVA	+ +	20 3	15 0
Duration of elimination 33 days	Leaf like villi some some high ridges	Slight mucosal changes	+	27 0	9 0
Duration of provocation 41 days	High ridges	Slight mucosal changes	+	27 0	15 2

[a] SVA subtotal villous atrophy PVA partial villous atrophy
– = within normal limits
+ = pathological change
+ + = pronounced pathological change

RESULTS

Initial biopsies

The initial proximal jejunal biopsies were performed as soon as possible after admission The initial biopsy of patients 1 and 2 could be studied 1 3 and 3 1 months respectively after withdrawal of cow s milk from the diet At this time the mucosa still showed partial or subtotal villous atrophy The surface epithelium displayed some changes, such as slight intra epithelial lymphocytic infiltration, but the height of the epithelial cells was not much lower than in healthy infants the range for healthy infants being 28 4–34 2 μm (7) (Table 3) Under the electron microscope the epithelial structure also appeared essentially normal in patient 2, except for some thickening of the basal lamina, whereas the specimen from patient 1 displayed some shortening and plumping of the microvilli as well as abnormal nuclei (Table 3) Patient 3 was the only one in whom a proximal jejunal biopsy was done before any treatment was given In light microscopy the specimen showed subtotal villous atrophy with a decreased cell height in the surface epithelium which was heavily infiltrated with lymphocytes and plasma cells as was the lamina propria (Fig 2) Electron micrographs displayed short microvilli occasionally fused at the bases (Fig 3) In some of these cells the nuclei had lost their perpendicular orientation to the surface and oc

...lectron microscope		
...hort ...bnormal ...icrovilli	Nuclei of abnormal shape and position	Thickening of basement membrane
+	+	−
+	+ +	+ +
	−	−
	−	+
...ot done	Not done	Not done
+	+ +	+ +
	+	+
+ +	+	+
	−	−
+	−	−

...ctional nuclei were amoeba like The epithelial basement lamina was unevenly thickened but without collagen fibres or other abnormal deposits (Fig 4)

Provocation with cow's milk

Patient 1 reacted rapidly The changes could already be seen under a light microscope in a biopsy specimen taken 20 hours after a single challenge with cow's milk The most striking change was the round cell infiltration into the surface epithelium and the lamina propria The height of the surface epithelial cells had also decreased (Table 3) Electron micrographs of this biopsy specimen showed shortening of the microvilli abnormality of nuclear shape and loss of perpendicular orientation of the nuclei and infiltration of lymphocytes in to the surface epithelium Some increase in the number of lysosomes was noticed in the apical part of the cells

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The withdrawal of cow's milk from the diet for about a month in patients 2 and 3 (from the initial situation in the latter) normalized the structure of the surface epithelium considerably in both light and electron microscopy (Table 3) The biopsy from patient 1 after a diet containing no cow's milk for 4.5 months displayed an almost normal epithelium in light and electron microscopy Some empty looking areas were encountered possibly due to a fixation artefact of the absorbing cells (Fig 9)



Fig 3 Apex of cells of the surface epithelium of patient No 3 before any treatment Note the short and in some cases basally fused microvilli The extensions of their internal filaments in the terminal web are frequently confluent at their tips (*arrow*) *M* = mitochondria *ER* = rough surfaced endoplasmic reticulum × 15 000

DISCUSSION

Changes identical with those present in coeliac disease (13 14 15 16 19 20 23 26) have been discovered before and in this study in the small intestinal mucosa of patients suffering from the malabsorption syndrome with cow's milk intolerance (4 7 8 10 21) In this study a challenge like that used to establish gluten toxicity in coeliac disease was employed to test whether cow's milk can immediately damage the small intestinal mucosa Provocation had to be performed in an early phase of the disease however because cow's milk intolerance itself is transient and disappears before the mucosa is normal This presented special difficulties systematic and repeated biopsies were not possible because of the young age and poor clinical condition of the patients

The results of this study show that a clinical reaction to cow's milk in these patients during the challenge period is accompanied by morphological changes in the small intestinal mucosa we therefore believe that the mucosal lesion is really induced by cow's milk Our results do not throw much light on the sequence of events leading to the morphological change In a biopsy specimen from a patient who reacted rapidly changes were visible only 20 hours after a single challenge In another patient who reacted slowly the changes could be seen in a specimen taken on the 4th day of challenge many days before clinical symptoms appeared Apparently the mucosal lesion precedes the clinical symptoms and with continuous challenge the morphological changes became more evident

Under the light microscope the jejunal picture during provocations in the present patients resembles that seen in coeliac disease At the ultrastructural level there are both similarities and differences Bayless et al (2) de

Fig 4 Basal lamina (*BL*) of patient No 3 before treatment Note the uneven thickening of the lamina The basal lamina of a capillary (*CA*) is also thickened 20 000



scribed increased lysosome formation at 4 5 hours after gluten feeding This we also observed in two of our patients Shmerling & Shiner (22) emphasized changes at 2 hours and 48 hours in the basement lamina after intra-duodenal gluten instillation in two children with coeliac disease We only saw such extensive alterations in the basement lamina in a patient reacting slowly after continuous feeding with the cow s milk formula for 38 days As regards the microvilli we found alterations in every biopsy after challenges Both Shmerling & Shiner (22) and Bayless et

Fig 5 The tip of a villus of patient No 2 after withdrawal of cow s milk for 3 1 months The surface epithelium is of nearly normal height but the polarity of the nuclei is somewhat disturbed and intraepithelial lymphocytes are still evident HE 900



al (2) emphasized the lack of microvillous alterations in their short term experiments On the other hand, the present long term provocations resemble the situation in active coeliac disease when microvillous alterations are common In all patients elimination of cow's milk improved both the clinical condition and the morphological changes of the mucosa Yardley et al (25) stated that in adult coeliacs after gluten withdrawal the epithelial cell height had improved within 6–10 days In contrast the return of villi usually requires sev

Fig 6 Flat surface epithelium from patient No 2 four days after the start of cow's milk challenge The epithelium is flattened and the nuclei have lost their elongated shape and perpendicular orientation to the surface Round cell infiltration is also evident The brush border is clearly visible HE ×900



Fig 7 Surface epithelium of patient No 2 after feeding with cow's milk for 33 days The microvilli are short and plump and in several instances fused at their bases Large collections of electrodense lysosomes are seen in the apical part of the epithelial cells (*arrows*) The epithelial nucleus (*N*) at the centre of the picture shows indentations and folding of the nuclear surface giving an amoeba like appearance ×7000

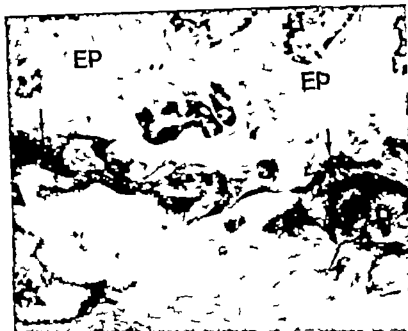


Fig 8 Basal lamina of the same biopsy as in Fig 7 Large accumulations of whirled collagen like fibres (*arrows*) are attached to the BL *EP* = epithelial cells ×7 600

eral months or more of gluten restriction In the present study one of the slowly reacting patients revealed the normal surface epithelial cell height and a clearly diminished intra epithelial inflammation after withdrawal of cow s milk for 33 days As regards the villous architecture one of the slowly reacting patients No 3 and the rapidly reacting patient No 1 showed only slight villous abnormality after elimination of cow s milk for 27 days and 4 5 months respectively In the latter case the height of the surface epithelial cells was also normal

Which fraction of cow s milk exerts the harmful effect on the small intestinal mucosa? We regard the protein part of cow s milk as responsible for this damage This theory is supported by our earlier studies in which we demonstrated that children with this syndrome were intolerant to protein fractions isolated from cow s milk (25) and by the work of Lin et al (11) In vitro studies have also suggested that cow s milk proteins such as α lactoglobulin and β lactalbumin are the agents responsible for jejunal mucosal damage (13) This would accord with the well known fact that a protein (gluten) causes the mucosal damage in coeliac disease Furthermore it has recently been reported that soya protein also causes small intestinal damage in rare patients who are already sensitive to cow s milk and have the malabsorption syndrome (1) Lactose is excluded as a cause because breast milk which was used as the elimination diet also contains this sugar

The malabsorption syndrome with cow s milk intolerance resembles coeliac disease not only in morphology but also in immunological features In both disorders elevation of serum IgA and antibodies to dietary proteins are seen in the active phase of the disease (6) Furthermore a similar strong local immunological reaction is seen in the jejunal mucosa of the patients in both conditions (17 18) This evidence suggests that immunological immaturity of the patient is causal in the pathogenesis of the malabsorption syndrome with cow s milk intolerance but the nature of its mechanism is still obscure

SUMMARY

Three infants in whom the malabsorption syndrome small intestinal mucosal damage and clinical cow s milk intolerance were



Fig 9 Surface epithelium of patient No 1 after weaning off cow's milk for 4.5 months. The structure of the epithelial cells is normal except for some empty looking areas (*arrows*) × 5 000

found, were challenged with cow's milk after initial treatment with breast milk. The small intestinal mucosa was investigated with light and electron microscopy both before and after provocation.

Clinically one patient reacted rapidly in a few hours and showed round cell infiltration in the surface epithelium and lamina propria 20 hours after a single challenge. Electron microscopy showed short microvilli, abnormal nuclei and thickened basement lamina in the surface epithelium. Two patients reacted slowly. One of them showed similar changes 4 days after commencement of the provocations, but the changes were much more evident 38 days later when symptoms were also apparent. At this time large accumulations of lysosomes in the apical part of the surface epithelial cell and marked thickening of the basal lamina with accumulations of whirly collagen fibres were detected. The third patient reacted in a milder way both clinically and morphologically. This study indicates that cow's milk, apparently through its protein fraction, may damage the surface epithelium of the small intestinal mucosa. These alterations during provocation periods resemble those found in coeliac disease during gluten provocation.

ACKNOWLEDGEMENTS

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COMPARATIVE MEASUREMENTS OF ENZYME ACTIVITIES AND 2,3 DIPHOSPHOGLYCERATE IN THE ERYTHROCYTES OF NEWBORNS WITH TRANSITORY HYPERBILIRUBINAEMIA

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Transitory hyperbilirubinaemia has been defined as a benign increased level of unconjugated serum bilirubin occurring in newborn infants

The cause of transitory hyperbilirubinaemia in the newborn has not been completely elucidated despite much work in this field (23) Delayed excretion of bilirubin due to deficiency of glucuronyltransferase no longer appears to be the sole explanation (8 14 25) Increased destruction of erythrocytes not due to isoimmunization, such as spherocytosis glucose 6 phosphate dehydrogenase and pyruvate kinase deficiency may also cause an increase in serum bilirubin levels (4 6 7 16 17)

In newborns the intraerythrocyte enzymes show a markedly higher activity with a wider standard deviation as compared to adults (19 27) Similarly increased activities were found in young adult red cells obtained from the upper layers of a centrifuged column of blood (21) Thus the higher activities of enzymes in newborns may be explained by a younger cell population This was confirmed by investigations of Zipurski (31) who found in five newborns a red cell life span from 50 to 105 days In children, where red cells show a life span of 50 days, more bilirubin will be produced than in other children In the following study the activities of seven enzymes were measured The results are compared to the serum bilirubin of these children As a result of these investigations 2 3-diphosphoglycerate was measured

The following enzymes were investigated 1 Phosphoglucose isomerase (EC 5 3 1 9) 2 Aldolase (EC 4 1 2 13) 3 Glyceraldehyde 3 phosphate dehydrogenase (EC 1 2 1 12) 4 3 Phosphoglycerate kinase (EC 2 7 2 3) 5 Pyruvate kinase (EC 2 7 1 40) 6 Lactic dehydrogenase (EC 1 1 1 27) 7 Glucose-6 phosphate dehydrogenase (EC 1 1 1 49)

MATERIALS AND METHODS

For this study term born infants were selected with uncomplicated obstetric history The average weight was 3 370 ± 387 g Each enzyme was studied in 37 cases 2 3 diphosphoglycerate in 20 cases Patients with serologic incompatibilities were not included in the study

Blood obtained by venipuncture was haemolysed for measuring enzyme activities according to Lohr & Waller (13) within 2 hours Determination of serum bilirubin was carried out as described by Jendrassik (10)

Methods of measuring enzyme activities phosphoglucose isomerase (15) aldolase (1) glyceraldehyde 3 phosphate dehydrogenase (3) 3 phosphoglycerate kinase (5 12) pyruvate kinase (9) lactic dehydrogenase (29) glucose 6-phosphate dehydrogenase (11)

All biochemical reagents were supplied by C. F Boehringer & Sons GmbH Germany All other reagent grade were obtained from E Merck AG Darmstadt Germany

Table 1 *Mean activities of enzymes and standard deviations regardless of serum bilirubin*

The results in the first column are compared with investigations of Witt et al (27) Enzyme activities in µmol/10[9] red cells min

	$n=32$	$n=10$
1 Phosphoglucose isomerase	2.2±0 9	2 1±1 1
2. Aldolase	0 25±0 09	0 16±0 03
3 Glyceraldehyde 3 phosphate dehydrogenase	5 3±2 2	4 5±0 9
4 3-Phosphoglycerate kinase	3 8±1 3	4 2±1 1
5 Pyruvate kinase	0 7±0 4	0 5±0 1
6 Lactic dehydrogenase	5 7±3 1	3 9±0 6
7 Glucose 6-phosphate dehydrogenase	0 36±0 11	0 38±0 09

2,3 Diphosphoglycerate was measured according to Schroter (22)

The results of the investigations were evaluated by regression analysis and determination of the *p* value

RESULTS

In Table 1 all enzyme activities without regard to serum bilirubin are summarized All enzymes except pyruvate kinase and glucose 6-phosphate dehydrogenase show a negative correlation to bilirubin In Table 2 the correlation coefficients and the *p*-values are shown Good *p*-values were found for phosphoglucose isomerase and glyceraldehyde 3 phosphate dehydrogenase although they are not above the 95 confidence limit The regression lines for these enzymes are shown in Figs 1 and 2

An unimpaired activity of glyceraldehyde 3 phosphate dehydrogenase is important for

Table 2 *Correlation coefficients and p values to serum bilirubin for the investigated enzymes* ($n=32$)

1 Phosphoglucose isomerase	−0 323	92 8
2 Aldolase	−0 163	61 0
3 Glyceraldehyde 3 phosphate dehydrogenase	−0 273	87 0
4 3 Phosphoglycerate kinase	−0 205	73 0
5 Pyruvate kinase	+0 187	68 0
6 Lactic dehydrogenase	−0 057	23 6
7 Glucose-6-phosphate dehydrogenase	+0 076	10 0

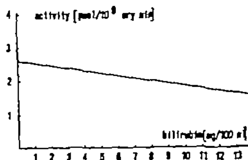


Fig 1 Activity of phosphoglucose isomerase and regression line in newborn infants with various serum bilirubin levels.

the intraerythrocyte 2 3-diphosphoglycerate level (30) So we decided to estimate 2 3-diphosphoglycerate in icteric and nonicteric children The statistic level is 57 µmol/10[11] red cells at zero and 32 µmol/10[11] red cells at 10 mg bilirubin/100 ml serum See Fig 3 ($r=-0\ 610$ p-value $=99\ 0$) The mean concentration regardless of bilirubin is 41 1 ± 18 4 µmol/10[11] red cells

DISCUSSION

In newborns red cell enzymes show an increased activity and a wider standard deviation as previously stated by other authors (19 27) This again indicates a younger red cell population since enzyme activities are

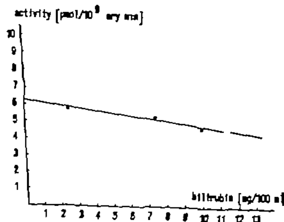


Fig 2 Activity of glyceraldehyde 3 phosphate dehydrogenase and regression line in newborn infants with various serum bilirubin levels

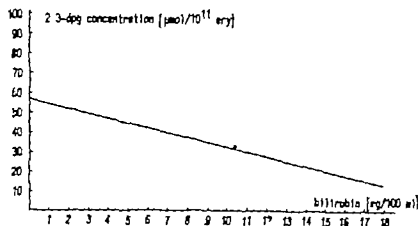


Fig 3 2 3 Diphosphoglycerate concentration and regression line in newborn infants with various serum bilirubin levels

in a similar range as in young adult red cells In Table 1 our results are compared to investigations of Witt et al (27) Glucose 6 phosphate dehydrogenase is well known to exhibit a definite increase in activity with decreasing age of the red cell (21) Fig 4 shows that there is no difference in activity in icteric and nonicteric children Thus there will be no difference with respect to red cell age In children with an elevated serum bilirubin statistical analysis indicates that several red cell enzymes have a lower activity The decrease in activity for phosphoglucose isomerase and glyceraldehyde 3 phosphate dehydrogenase is striking where *p* values of 92 and 87% were found (Figs 1 and 2) An even better correlation would probably be found if bilirubin production and excretion could be measured separately In addition the bilirubin concentration in serum is dependent upon many other factors such as age of mother blood volume of the child etc (26 28) If all these parameters could be measured *p* values above the 95% confidence level would probably be found Therefore we consider the momentary bilirubin level to be a rather bad parameter for a changed intraerythrocyte metabolism Earlier investigations of red cell enzymes were carried out by Stave (24) who found a slightly higher activity in icteric children These investigations however were made in premature infants aged four to six days At this age red cell enzymes show a rapid decline in activity up to 80% (24) Thus the different results cannot be compared to each other because our infants were all examined on third day of life

Reinauer showed that glyceraldehyde 3 phosphate dehydrogenase is the limitating enzyme of glycolysis at a physiological pH value (20) The decreased activity of this enzyme in icteric children therefore indicates a reduced glycolysis A similar assumption was already made by Oski (18) who supposed in erythrocytes of newborns a reduced glucose consumption as compared to young red cells of adults Bilirubin however was not considered in his study On the other hand glyceraldehyde 3 phosphate dehydrogenase may regulate the intraerythrocyte 2,3 diphosphoglycerate level When the enzyme is inactivated completely by iodoacetate 2 3-diphosphoglycerate shows a rapid and complete fall in concentration (30) Because of the importance of 2 3 diphosphoglycerate for the position of the oxygen dissociation curve (2) and

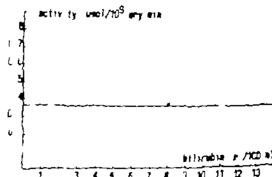


Fig 4 Activity of glucose 6-phosphate dehydrogenase and regression line in newborn infants with various serum bilirubin levels

for the metabolism of activated phosphorylates 2 3-diphosphoglycerate was correlated to bilirubin We found a significant decrease in children with an elevated serum bilirubin Without regard to serum bilirubin the average level is in the same range as described by Schroter (22) The different *p*-values for glyceraldehyde 3 phosphate dehydrogenase and 2 3-diphosphoglycerate may be explained by the diphosphoglyceromutase which produces 2 3-diphosphoglycerate from 1 3-diphosphoglycerate

The reduced 2 3-diphosphoglycerate levels in the erythrocytes from newborns suffering from transitory hyperbilirubinaemia indicate an altered red cell metabolism and a shift to the left of the oxygen dissociation curve

SUMMARY

The activities of various enzymes in the erythrocytes of newborn infants suffering from transitory hyperbilirubinaemia have been studied Phosphoglucose isomerase (*p* value = 92 *) and glyceraldehyde 3 phosphate dehydrogenase (*p*-value = 87 %) show a decrease in icteric children These *p*-values were regarded as sufficient for further investigations because bilirubin level is influenced by many different factors Because of the importance of glyceraldehyde 3 phosphate dehydrogenase for the intraerythrocyte 2 3-diphosphoglycerate level 2 3-diphosphoglycerate was measured and correlated to serum bilirubin A steady decline in icteric children is noted (*p*-value = 99)

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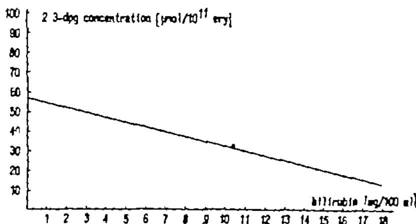


Fig 3 2 3 Diphosphoglycerate concentration and regression line in newborn infants with various serum bilirubin levels

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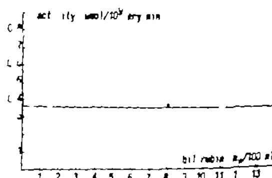


Fig 4 Activity of glucose 6-phosphate dehydrogenase and regression line in newborn infants with various serum bilirubin levels

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THE RE EMERGENCE OF EARLY CONGENITAL SYPHILIS

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An increasing incidence of acquired syphilis has been reported in Western countries (1 9) as well as in Singapore (6) It is therefore not surprising that congenital syphilis is again becoming more frequent Congenital syphilis has different forms of presentation the severe form presenting at birth is rare (7) and denotes a grave prognosis (1) It is the purpose of this paper to present an analysis of ten consecutive cases of early congenital syphilis diagnosed at birth at the Kandang Kerbau Hospital Singapore and to emphasize a feature that has usually been overlooked or only received very little attention in the past It is perhaps timely to stress that early congenital syphilis is not as rare at present as is sometimes thought

MATERIAL AND METHOD

The 10 consecutive cases of neonatal syphilis were encountered by the author in the Kandang Kerbau Hospital Singapore where over 30 000 deliveries occur annually Infants suspected to be suffering from congenital syphilis were carefully examined clinically initially and further investigated where deemed necessary these included those infants with mothers who had positive serological tests for syphilis (STS) though the majority of these mothers had been treated Investigations included a complete haematological examination serological tests employing the Venereal Disease Research Laboratory (VDRL) and the Fluorescent Treponemal Antibody Absorption (FTA ABS) tests immunoglobulin estimation by a modified form of the radial immunodiffusion method (5) radiology of the skeletal system and where feasible direct examination for treponema by the dark ground technique Where death occurred a complete autopsy was performed

The survivors were followed up regularly after discharge and their progress noted

RESULTS

Ten consecutive cases of neonatal congenital syphilis diagnosed by the author are presented for analysis

Clinical features

The maternal age ranged from 20 to 31 years with a mean of 27 years the birth rank of the affected infants showed an equally wide variation (Table 1) In 8 the father was the source of infection and in 2 the mother the fathers having negative VDRL and FTA ABS tests

All the infants were small either premature or small for date (Table 2) the placentae were much larger and heavier than would be normally expected the infant–placenta weight ratio ranging from 1 5 1 to 4 1

Hepatosplenomegaly was a constant feature and was largely responsible for the distended abdomen presented by the majority of the infants Four of the infants (Cases 4–7) presented with pallor bloated abdomen and oedema features characteristic of hydrops fetalis indeed because of this manner of presentation the possibility of congenital syphilis was not even entertained by the obstetricians[1]

Raw extremities oozing serosanguinous

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Key words Bilirubin red cell enzymes red cell metabolism oxygen dissociation curve

Table 3 *Laboratory and radiological findings*

	Cases							
	1	2	3	4	7	8	9	10
Haemoglobin level (g/100 ml)	12 0	9 9	10 4	9 1	9 4	11 8	15 5	16 4
Reticulocytosis ( )	7 0	1 0	1 0	—	4 0	7 0	7 0	4 0
Normoblasts (per mm)	—	15 300	—	5 000	7 300	—	7 000	—
Leucocytes (per mm)	27 400	15 300	36 000	32 000	24 100	9 900	24 500	6 900
Platelets (per mm)	85 000	140 000	100 000	80 000	400 000	140 000	30 000	70 000
Mother s VDRL titre	1 32	1 32	1 32	1 64	1 8	1 32	1 8	+
Baby s VDRL titre	1 32	1 128	1 128	1 128	1 32	1 128	1 64	+
Mother s FTA-ABS	+	+	+	+	+	+	+	+
Baby s FTA ABS	+	+	+	+	+	+	+	+
IgM (mg/100 ml)	10 4	94	78	132	132	ND	146	ND
IgA (mg 100 ml)	14 8	61 6	52	24	—	ND	46	ND
IgG (mg/100 ml)	1 459	840	1 195	470	428	ND	680	ND
Radiology								
Metaphysitis	+	+	+	+	+	+	+	+
Osteomyelitis	–	–	+	–	+	–	–	–

ND not estimated

being only 10 3 g per 100 ml this was much less than the levels of 15 5 and 18 3 g per 100 ml in the 2 infants treated prenatally Reticulocytosis or normoblastaemia was present in the majority as was thrombocytopenia and leucocytosis

There was no evidence of Rhesus or ABO isoimmunization or alpha thalassaemia

Serology and immunoglobulins (Ig)

The mothers of all the affected infants had positive STS on testing In 2 infants (Cases 5 and 6) the amount of blood obtained was only sufficient for serological tests the infants dying shortly after delivery FTA ABS and VDRL tests were positive the latter demonstrating a titre of 1 256 in both infants compared with a maternal titre of 1 16 in one (Case 5) and 1 32 in the other (Case 6) newborn titres higher by sixteen times and eight times respectively In 5 other infants (Table 3) the infant titres were four times higher compared with those of the mothers There was also significantly raised IgM levels in all these infants as well as IgA except for one infant (Case 7) in whom the IgA was absent One infant presenting with marked pemphigus (Case 1) did not have a raised IgM

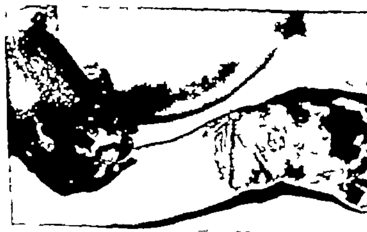


Fig 1 Raw extremities oozing serosanguinous fluid teeming with treponemes (Case 1 at 4 days of age)

Table 1 *Clinical features*

	Cases									
	1	2	3	4	5	6	7	8	9[a]	10[a]
Sex	F	M	F	F	F	M	F	M	M	F
Birth rank	2	10	6	3	3	3	6	8	2	4
Maternal age	20	30	28	29	21	25	30	30	26	31
Previous abortion	—	—	—	1	—	—	1	1	—	1
Gestation (weeks)	41	40	33	45	28	32	38	38	38	36
Birth weight (g)	1 750	1 670	1 815	1 843	990	1 765	2 630	2 650	2 450	1 875
Placental wt (g)	500	420	480	830	700	—	650	—	—	—
General condition	Fair	Poor	Fair	Poor	Moribund	Moribund	Poor	Good	Good	Good
Hepatomegaly (>2 5 cm)	+	+	+	+	+	+	+	+	+	+
Splenomegaly (>1 0 cm)	+	+	+	+	+	+	+	+	+	+
Hydrops fetalis	–	–	–	+	+	+	+	–	–	–
Raw extremities	+	+	+	+	+	–	–	–	–	–
Snuffles	–	–	+	+	–	–	–	–	–	–
Outcome	died	died	alive	died	died	died	alive	alive	alive	alive

Mother had syphilotherapy 1 month before delivery (600 000 units of procaine penicillin in oil with 2 alum. monosterate daily for 15 days)

fluid (Fig 1) were present at birth in 5 infants and was the most striking feature raw areas were also present in both ears in one infant (Case 3) who also had serosanguinous discharge from the nostrils The serosanguinous fluid from the extremities as well as from the ears and the nostrils were found to be teeming with treponemae on dark ground examination The skin in all the cases was normal except in those with hydrops fetalis in whom they were thin shiny and oedematous

On the third day of life skin lesions in the form of a light red circle enclosing a central area of deeper red appeared in one infant (Case 1) Initially faint the lesions became progressively darker and larger (Fig 2) An other infant (Case 3) presented with paralysis of both arms on the fourth day of life, movements were possible but kept to the minimum and handling of the infant resulted in crying Careful clinical examination and X rays revealed the underlying cause (Fig 3)

The general condition of the last 3 infants was good at delivery hepatosplenomegaly being the sole presentation investigations of the hepatosplenomegaly revealed the diagnosis In 2 of these infants (Cases 9 and 10) the mothers had syphilotherapy in the last month of pregnancy

Table 2 *Frequency of clinical manifestations*

Manifestation	Frequency ( )
Poor general condition	90
Low birth weight (<2 5 kg)	80
High placental infant wt ratio (<1 5)	100 (6)
Hepatomegaly	100
Splenomegaly	100
Raw extremities	50
Hydrops fetalis	40
Snuffles	20

Figure in parentheses indicates the actual no with the relevant information

Radiography

In all the 9 infants X rayed osseous lesions ranging from submetaphyseal radiolucent bands to severe osteomyelitis and periostitis (Fig 4) were demonstrated Radiological healing of the lesions occurred within 3 weeks in Case 9 a treated case but in Case 3 with severe osteomyelitis and fracture healing was complete only after 7 weeks though there was some callus still at the fracture site 2 weeks later the callus had completely disappeared

Haematologic findings

Anaemia was present in all the untreated cases (Table 3) the mean haemoglobin level

Table 3 *Laboratory and radiological findings*

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Serology and immunoglobulins (Ig)

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Fig 1 Raw extremities oozing serosanguinous fluid teeming with treponemae (Case 1 at 4 days of age)



Fig 2 Macular lesion in form of circle enclosing a spot of similar but darker red (Case 1)



Fig 3 Fracture in ulna giving rise to pseudoparalysis (Case 3) Complete healing and remodelling occurred

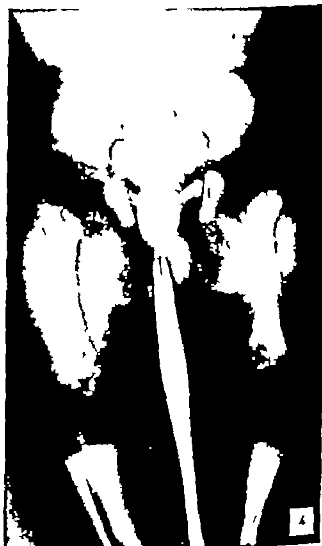


Fig 4 Severe osteomyelitis and periostitis of lower limb bones (Case 3)

level nor was her VDRL titre elevated above that of her mother

Progress

Five of the infants died 2 shortly after delivery and 2 more (Cases 2 and 4) in the second day of life all the 4 infants were in very poor condition from birth One infant (Case 1) survived for 1 week being initially well progressive hepatosplenomegaly and macular skin lesions occurred before she finally succumbed

One infant (Case 3) with severe skin and bone lesions with resulting pseudoparalysis survived after intensive therapy consisting of crystalline benzylpenicilline intramuscularly 12 hourly for 1 week (total dosage 805 000 units—slightly more than 400 000 units per kg body weight) and careful nursing the extremities healed by 2 weeks and the bones by 7 weeks Another infant (Case 7) with marked hydrops fetalis subsequently bled a little from the cord with blood transfusion and immediate intensive therapy he survived the oedema and ascites subsiding rapidly within the next 2 days The other 3 infants made uneventful recoveries 2 of these had prenatal syphilotherapy but treatment was instituted (as above) as an added precaution The hepatosplenomegaly in all survivors persisted for more than 2 months

Subsequent progress of the surviving infants has been satisfactory with normal physical and mental development

Autopsy

Gross hepatosplenomegaly was demonstrated in all the 5 infants who died ascites was present in the three with hydrops Increased numbers of haemopoietic foci were present in the liver and spleen the former also demonstrated pericellular fibrosis in 2 infants (Cases 5 and 6) The lungs in 2 infants (Cases 1 and 6) presented with patchy interstitial fibrosis and increased leucocytic infiltration There was interstitial fibrosis in the pancreas in all cases Treponemae were demonstrated in the lungs and the pancreas The heart and kidneys appeared normal except for mononuclear infiltration of the latter

DISCUSSION

Clinical manifestations of congenital syphilis are infrequent before the third week of life (10) When obvious at birth the prognosis is usually poor (1) This seems to be borne out in this series as 5 of the infants died Only 3 of the infants survived besides the two prenatally treated cases However with early intensive treatment a better survival rate is possible as evidenced by the survival of one infant (Case 7) with severe hydrops in whom blood transfusion and intensive therapy were started shortly after delivery Treatment in all the severe cases consisted of crystalline benzylpenicillin administered intramuscularly 12 hourly over a week with a total dosage of about 400 000 units per kg body weight It was thought that the high blood levels rapidly achieved with crystalline benzylpenicillin would rapidly control the infection render the treponemae harmless and the infant non infective The usual procaine penicillin (200 000–300 000 Units per kg body weight) would most likely be just as effective in the treatment of this condition since effective treatment does not need high levels of penicillin in the blood but perhaps the effect of rendering the treponemae harmless might take a longer time

None of the infants presented with the emaciated wizened appearance which is often a characteristic of severe congenital syphilis (8) Instead 4 of the infants presented with hydrops fetalis a feature that has only been mentioned in passing before and consequently often overlooked besides the reports of this association preceded the availability of methods capable of detecting isoimmunization to date only one case of syphilitic hydrops fetalis has been reported in whom isoimmunization and alpha thalassaemia has been excluded (2) In the present four cases these



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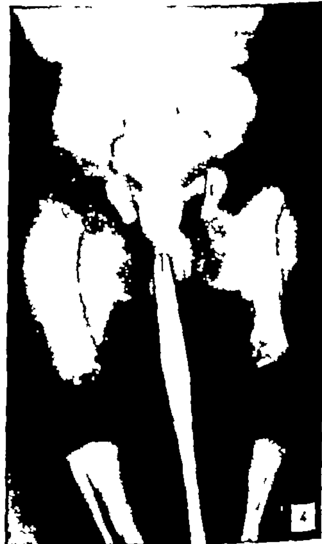


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causes have also been excluded The placentae as in hydrops fetalis from other causes were large and oedematous (3) The pathogenesis of hydrops is still obscure (3) though many factors have been implicated undoubtedly anaemia and hypoproteinaemia play a vital role With the present cases these factors would have played a major role in causing the hydrops That four out of ten consecutive cases of early congenital syphilis should present with hydrops indicates that this mode of presentation is perhaps commoner than has been generally thought Since adult acquired syphilis is again on the increase it would seem advisable that in all infants presenting with hydrops fetalis, congenital syphilis should be specifically excluded

The present experience indicates that hydrops fetalis caused by congenital syphilis need not be invariably fatal with early diagnosis and intensive therapy including blood transfusion survival can sometimes occur This would seem to be the first report of survival in syphilitic hydrops fetalis

Presence of raw areas or bullae at the extremities especially in a small infant is a very helpful feature in the diagnosis of congenital syphilis since any pemphigus present at birth is always syphilitic in origin (7) in the present series this feature contributed to the early diagnosis in the infants presenting with it Treponemae were observed on dark ground examination from the serosanguinous discharge

The last 2 infants (Cases 9 and 10) though having congenital syphilis were probably cured of the condition by prenatal treatment of the mothers early treatment of the mother during pregnancy prevents congenital syphilis as the treponema cannot cross the placental barrier before the fourth or fifth month of gestation (4 7) Late treatment (in pregnancy) does not prevent fetal infection but cures it (9) as evidenced by these two cases The infants were cured of the illness one month before delivery and the lesion were already healing though the stigmas of the infection were still present in the form of hepatosplenomegaly bone lesions elevated IgM and positive VDRL four times higher than the maternal level This would explain the rapid recovery of the bone lesions The relatively high haemoglobin levels of these 2 infants is a notable feature It is possible that the eradication of the infection enables the fetus to continue developing after the initial 'setback this would explain the still subnormal haemoglobin level as well as the thrombocytopenia However, it has been shown that treatment in the neonatal period does not prevent the anaemia being progressive (11)

The early diagnosis of neonatal syphilis can be very difficult Only syphilitic pemphigus is characteristic It is however a condition to be kept in mind in any infant presenting with the features summarized in Table 2, this becomes even more relevant where there is a positive maternal STS or a past history of abortion Hydrops fetalis too should arouse the suspicion of congenital syphilis especially in the absence of Rh or ABO isoimmunization In an era of increasing incidence of acquired syphilis among the young childbearing age group the possibility of congenital syphilis should always be kept in mind

Prevention of the occurrence of congenital syphilis would seem to be a more effective measure Routine STS should be performed in all pregnant females immediate treatment should be administered to those found to be suffering from syphilis This simple procedure would help greatly towards eradicating congenital syphilis

SUMMARY

Ten infants with early congenital syphilis are presented Hepatosplenomegaly is a marked feature in all the infants The majority of them were of low birth weight Syphilitic pemphigus was present in 5 and hydrops fetalis in 4

Five infants survived including one with

hydrops fetalis Early diagnosis and intensive therapy are necessary for survival A high index of suspicion is required for early and more frequent diagnosis of this condition

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ADRENAL GLUCOCORTICOID FUNCTION IN ACUTE VIRAL INFECTIONS IN CHILDREN

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Based on clinical observations, some acute viral infections produce alterations simulating those of hyperadrenocorticism (5 7 12 14)

Few laboratory data are available concerning the adrenocortical function during acute viral infections In measles estimations of the urinary 17 OHCS were done in a small number of cases and the results were contradictory (1 16) Plasma glucocorticoids were not studied in uncomplicated cases of chicken pox Cortisol production rates were estimated in a very few cases of chicken pox and rubella encephalopathy (21) Some of these cases showed a rise in the cortisol production rate while in other cases this was within normal limits

There are no data available about adrenocortical function during mumps while minimal work has been done on adrenocortical function in polio myelitis in adults (8) Moreover both the adrenocortical reserve and the pituitary ACTH capacity were not studied in any of the acute viral infections

The aim of the present work is (1) To study the hypothalamic pituitary adrenal system during certain acute viral infections (2) From these studies information may be obtained as regards the need for corticosteroid therapy in these viral infections

MATERIAL AND METHODS

The following groups of infants and children were investigated

I Group of normal children

Forty three normal healthy children were selected from the Child Welfare Centre and healthy siblings of inpatients in the Alexandria University Children's Hospital All the children were hospitalized Their ages ranged from 2 months to 13 years

These normal children served as the control group Estimations of plasma 17 OHCS were done in the whole group The ACTH test was carried out in 13 cases The metyrapone test was done in 10 children

II Group of children with viral infections

1 *Group of measles* Twenty six cases of measles were studied 8 males and 18 females Their ages ranged between 8 months and 6 years These cases were classified into mild moderate severe and complicated cases according to the general condition of the patient height of temperature density and spread of the rash and complications

In the whole group follow up estimations of plasma 17 OHCS levels were done The ACTH test was carried out in 12 cases

2 *Group of chicken pox* Thirty seven cases of chicken pox were studied 21 boys and 16 girls Their ages ranged between 16 days and 6 1 2 years

These cases were classified into mild moderate severe and complicated according to the general condition height of temperature density and extent of the rash and presence or absence of complications

Plasma 17 OHCS were estimated on successive days during the disease and convalescent period The ACTH test was done in 1_ cases The Metyrapone test was done in 5 patients

3 *Group of mumps* Forty cases were studied 26 boys and 14 girls Their ages ranged from 2 to 10 years

The cases were classified into mild moderate severe and complicated according to the general condition height of temperature size and extent of the glandular swelling and presence or absence of complications

Follow up samples for estimations of plasma 17

Table 1 *Plasma 17 OHCS levels according to the stage of measles (µg/100 ml)*

Stage	No of cases	Mean	S D
Catarrh	4	72 5	41 5
First ds rash	14	60 0	36 3
Second 2 ds rash	15	53 0	34 8
Post-erupt	6	30 0	25 0

OHCS were taken The ACTH test was done in 10 patients The Metyrapone test was carried out in 11 children

4 *Group of poliomyelitis* Nine cases of acute poliomyelitis with recent paralysis affecting the lower and or upper extremities were studied within 3 days of the onset of paralysis Their ages ranged between 8 months and 2 years

Plasma 17 OHCS were estimated in the whole group The ACTH test was done in 6 cases

METHODS

Study of the pituitary and adrenocortical glucocorticoid function was carried out by determination of plasma 17-OHCS levels using the modified Porter Silber method by Peterson et al (20) adrenocortical reserve by the 4 hour intramuscular ACTH test (22) and pituitary ACTH reserve by the oral metyrapone test (10 15)

Table 2 *Plasma 17 OHCS levels according to the stage of chicken pox (µg/100 ml)*

Stage (days of rash)	No of cases	Mean	S D
2nd	8	32 25	22 32
3rd	12	51 00	21 84
4th	14	45 30	29 98
5th 10th	11	36 00	25 98
11th	6	25 15	10 20

Table 3 *Plasma 17 OHCS levels for the mumps patients according to the stage of the disease (µg/100 ml)*

Stage of the disease	No of cases	Mean	S D
2 3 days of SW	13	31 54	12 96
4 days of SW	18	37 50	21 90
5 days of SW	9	21 11	12 44
6–10 days of SW	11	21 36	16 58
After 2 weeks	3	11 00	8 18

SW = G

All blood samples for the estimation of plasma 17 OHCS were taken under basal conditions (8–9 a m fasting and not under stress other than the disease itself)

Metabolic studies were made of hepatic and renal functions including blood urea thymol and zinc sulphate turbidity serum proteins and A/G ratio serum bilirubin and study of the metabolism of exogenous intravenously administred hydrocortisone (using the rapid intravenous hydrocortisone hemisuccinate method (17)) Other routine investigations as complete blood picture urine stools and CSF examination were performed

RESULTS

I *Healthy children*

The 17 OHCS levels among normal healthy children had an overall mean of 12 44 ± 7 27 µg/100 ml

In response to ACTH intramuscular injections in 13 normal subjects a mean rise of 42 8 ± 46 0 µg/100 ml was obtained This rise is significant at the 1 level using the paired *t* test (t = 12 10) The relative rise compared with the basal level had a geometric mean of 2 97 folds

The ingestion of metyrapone in 10 children caused a mean absolute rise in the 17 OHCS level of 25 5 ± 17 32 µg/100 ml This rise is significant at the 1 level using the paired *t* test (t = 14 72) The geometric mean of the relative rise reached 1 98 folds of the basal level

II *Measles group*

The levels of plasma 17 OHCS at all stages of measles were significantly higher than normal controls (Table 1) The mean level during the catarrhal stage is significantly higher than that of the eruptive and the post eruptive stages

There was no significant difference between the levels of severe and mild to moderate cases except during the catarrhal stage In severe cases with complications the mean plasma 17 OHCS levels were significantly higher than in severe and mild to-moderate cases throughout the duration of the disease

The ACTH test (done in 12 cases) showed an average increase of 2 17 folds The mean

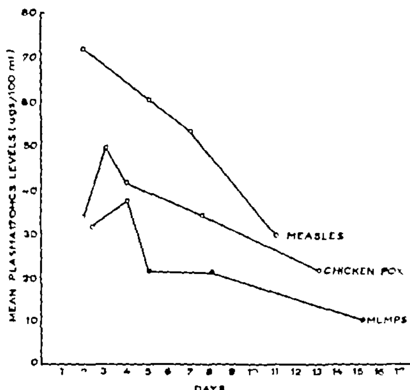


Fig. 1 Plasma 17 OCHS levels during viral infections

absolute rise of plasma 17 OHCS was 62 5 ± 38 5 µg/100 which is significant at the 1 % level ($t = 19\ 48$) This mean absolute rise is not significantly different from that obtained in the healthy control group ($t = 1\ 158$)

III *Chicken pox group*

There is a significant rise in the mean plasma 17 OHCS levels during all stages of the disease (Table 2) The mean plasma 17 OHCS levels are higher in severe complicated and severe cases than in mild to moderate ones

The ACTH test (done in 12 cases) resulted on the average in a 2 37 fold rise The mean absolute rise was 39 ± 23 6 µg/100 ml This value is significantly different from zero at the 1 % level ($t = 19\ 83$) The mean rise is not significantly different from that of the healthy group ($t = 1$)

The Metyrapone test (done in 5 cases) induced on average a 1 78 fold rise The mean absolute rise was 18 6 ± 16 9 µg/100 ml This value is significantly different from zero at the 1 % level ($t = 5\ 502$) This result is not significantly different from that of healthy children at the 5 % level ($t = 1$)

IV *Mumps group*

Throughout the first 2 weeks of glandular swelling the plasma 17 OHCS levels were significantly higher than normal at the 1 % level (Table 3) The mean plasma 17 OHCS levels were higher in severe complicated and severe than in mild to moderate cases throughout the whole illness

The ACTH test (done in 10 patients) showed an average rise of 2 66 folds in the plasma 17 OHCS levels The average absolute rise was 40 1 ± 30 0 µg/100 ml This rise is significant at the 1 level ($t = 13\ 33$) This value does not differ significantly from that of the healthy controls ($t = 1$)

On the other hand the response to Metyrapone test (done on 11 patients) resulted in an average rise of 2 3 folds The mean absolute rise is 22 6 ± 13 9 µg/100 ml which is also not significantly different from that of the control group ($t = 1$)

V *The poliomyelitis group*

The group of 9 patients with acute poliomyelitis gave a mean plasma 17 OHCS level of 26 6 ± 14 8 µg/100 ml This value is signifi

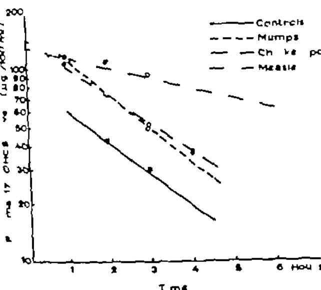


Fig 2 Hydrocortisone disappearance rate in the groups studied

cantly greater than that of the healthy group at the 1 level ($t = 2.73$)

The average rise after ACTH for 6 patients was 2.05 folds The mean absolute increase in the 17 OHCS was 28.4 ± 15.8 μg/100 ml This rise is significant at the 1 level ($t = 12.40$) This mean rise does not differ significantly from that observed in the control group ($t = 1$)

VI *Disappearance rate of exogenous hydrocortisone*

This phenomenon was studied over a 6-hour period in 4 groups namely 5 healthy controls 5 mumps 5 chicken pox and 5 measles patients The results are plotted on a semilogarithmic scale in Fig 2 The disappearance rates for mumps and chicken pox are more or less parallel with that of healthy controls The rate for the measles group however is slower than that of controls denoting a prolongation in the biological half life of the exogenous hydrocortisone

DISCUSSION

The present study of infants and children with acute viral infections showed a significant rise in plasma 17-OHCS levels in general However this rise is more marked in measles less in chicken pox still less in mumps while the least rise was found in poliomyelitis (Fig 1) It was generally observed that the mean plasma 17 OHCS levels were higher in severe and complicated cases than in mild to-moderate and non complicated cases in each of the studied groups with acute viral infections

Few data about the adrenocortical function during acute viral infections are available Lorenz & Rossipal (16) found a significant rise of urinary 17 OHCS during the prodromal and early eruptive state of measles Aceto and associates (1) reported almost normal levels of urinary 17-OHCS and cortisol production rates in man in non-complicated cases of measles and chicken pox Sherman and co-workers (21) reported high rates of cortisol production in fatal cases of german measles and chicken pox encephalopathy while the rates were normal in survivors with milder form of encephalopathy Migeon and associates (19) found a three fold rise in urinary 17 OHCS levels in a group of uncomplicated viral meningo-encephalitis

The high levels of plasma 17-OHCS obtained in this study of children with some acute viral infections may be the result of the stress imposed by the infection the febrile reaction

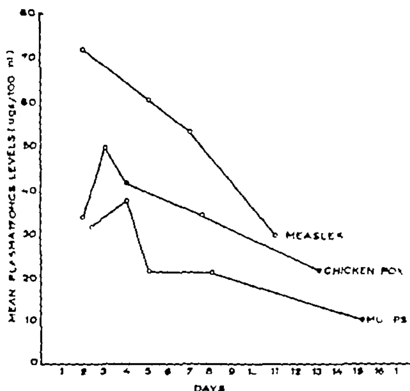


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fections However it seems reasonable to advise the administration of corticosteroids during the course of acute viral infections under the following circumstances

1 Children with definite adrenocortical insufficiency as in Addison s disease and the adrenogenital syndrome and following a prolonged course of corticosteroid administration in pharmacological doses

2 In children with acute overwhelming infections with shock or other complications such as haemorrhagic chicken pox or measles or vaccinia meningo encephalitis pneumonia or thrombocytopenia corticosteroids therapy is advisable

3 In children receiving corticosteroids in pharmacological doses for long periods as in rheumatic fever rheumatoid arthritis leukemia Hodgkin s and nephrosis the drug should not be stopped but the dose must be diminished to two or three times the physiological requirements when there is evidence of viral infection

SUMMARY

The hypothalamic pituitary adrenal system was studied in normal Egyptian children and in groups of children with some common viral infections including measles chicken pox mumps and poliomyelitis Results showed a significant rise in plasma 17 OHCS levels in all acute viral infections ACTH and metyrapone tests revealed that the hypothalamic pituitary adrenal axis was functioning adequately The value of corticosteroids in the management of acute viral infections is discussed in the light of our clinical and laboratory findings

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and/or deranged hepatic conjugation or renal excretion of glucocorticoids (2, 3 18)

There was no definite correlation between the height of temperature and the magnitude of rise in plasma 17 OHCS Higher levels of plasma glucocorticoids were obtained while the temperature was subsiding as on the second and the third days of chicken pox eruption In mumps many cases showed significant rise in plasma 17 OHCS in the presence of slight or no rise of temperature Similar results were obtained with pyrogen administration experiments in humans and animals (11 18) On the other hand, normal plasma cortisol level was found with etiocholanolone induced fever (13)

The results of the disappearance rate of *exogenous hydrocortisone (Fig 2) as well as* of the other tests done in this study for the assessment of hepatic and renal function were within normal limits during chicken pox and mumps infections In measles there was a prolongation of the biological half time of exogenous hydrocortisone i e a slowing of its disappearance rate This might be due to disturbance of hepatic degradation and/or renal excretion of glucocorticoids during this disease This might partially explain the higher plasma 17 OHCS levels obtained in measles than other acute viral infections However the results of the other tests done for hepatic and renal functions in measles were almost normal

On the basis of our results and most of those obtained by previous workers it is clear that the acute viral infections studied are stressful diseases sufficient to stimulate the hypothalamic pituitary adrenal axis resulting in an increased production of adrenocortical glucocorticoids The degree and the duration of elevation in plasma 17 OHCS levels varied from one disease to another and from one case to another within the same disease This would appear to depend on the individual himself the severity of the disease as a stress and the duration of this stress

The higher plasma 17 OHCS levels observed in severe and complicated cases of acute viral infections might be explained by the severity of the illness as a stress The further rise in plasma glucocorticoid levels with the occurrence of complications in some cases of viral infections, with already high levels of plasma glucocorticoid provides an evidence that both pituitary and adrenocortical reserves are still sufficient and not exhausted by these infections The significant rise in plasma glucocorticoid levels in response to ACTH stimulation in the studied cases of each disease supports this view

The metyrapone test carried out in some cases of chicken pox and mumps showed a significant response of the pituitary adrenal axis during these infections The response was not significantly different from that obtained in healthy children

The results obtained with the ACTH and metyrapone test in the present study revealed that the hypothalamic pituitary adrenal axis was adequately functioning and not exhausted even during severe cases of acute viral infections and that both the pituitary and adrenocortical reserves were intact

The value of the adrenal corticosteroids and corticotropin in the management of acute viral infections is still controversial despite extensive studies in experimental animals and humans (9 25) The use of these hormones has been suggested to treat severe or overwhelming infections to prevent the occurrence of complications and to manage the shock state which might occur during the course of the disease (4 6 23 24)

Our results showed that on clinical grounds all patients including those whose illness was severe or complicated recovered completely without corticosteroid administration and without showing any clinical evidence of adrenocortical insufficiency On laboratory grounds they showed a significant rise in glucocorticoid secretion which was almost proportionate to the severity of the illness and complications All the cases responded efficiently to ACTH and metyrapone administration It thus seems that glucocorticoids are not advisable as a part of the therapeutic regimen in acute viral in

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LOWE'S SYNDROME

Absence of Amino Acid Transport Defect in Cultured Fibroblasts

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Groth & Rosenberg (3) recently reported absence of transport defect in cultured fibroblasts of patients with two kidney and intestine amino acid transport disorders cystinuria and Hartnup disease We wish to report the results of our studies of the uptake of L lysine and glycine by skin fibroblasts cultured from a patient with another hereditary transport disorder Lowe's syndrome (case 2 in Lowe's (5) original description of the disease) as compared with a healthy control

MATERIAL AND METHODS

Transport was measured using a modification of Foster & Pardee's (2) technique as further modified by Isselbacher (4) Cells from the 2 individuals were grown in Eagle's minimal essential medium plus non essential amino acids (GIBCo) containing 15% fetal calf serum (Gray Industries) and cultured at 37 C under a 95% air 5% CO atmosphere in 8 100 mm Petri dishes divided in quadrants containing phosphate buffered saline (PBS) 2 5 10 cells were inoculated into each compartment containing sterile glass coverslips In order to study amino acid uptake the medium was aspirated 5 days later and the compartments were washed twice with PBS 2 ml PBS was then added to each quadrant along with 0 1 ml (2 0 mM) of L lysine ¹⁴C (spec act 312 mCi/mM New England Nuclear Boston Mass) After a further 1 hour incubation at 37 the PBS was aspirated 1 0 ml of a 0 25% trypsin solution added to one coverslip (non radioactive) from each dish and after stopping the trypsinization with 2 0 ml of serum-containing medium the cells counted in a Coulter counter

The labelled coverslips were thoroughly rinsed in PBS dried overnight and counted in toluene PPO POPOP solution in a Beckman LS 230 counting system

RESULTS

Average uptakes ± standard deviations were as follows for the one hour labelling expressed in cpm/cell

	Glycine CPM (cell [illegible])	L lysine CPM (cell [illegible])
Control	1 438 ±5[illegible]0 (n=5)	425 ±179 (n=5)
Lowe's	890 ±101 (n=6) N S ($P > 0.1$)	431 ±29 (n=6) N S

n = number of coverslips counted

DISCUSSION

Although in addition to the renal aminoaciduria an amino acid transport defect for lysine and arginine has been shown in the intestine of patients with Lowe's syndrome (1) no studies of other tissues have been available Our studies indicate that skin fibroblasts cultured from this patient with Lowe's syndrome show no defect in the transport of lysine or glycine Although there is a suggestion that the Lowe's fibroblasts took up less glycine than the control several values and the standard deviations overlap and the degree of difference is not significant ($p > 0.1$) To date no transport defect has been demonstrated in fibroblasts obtained from individuals affected by transport

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THE TRANSCAPILLARY ESCAPE RATE OF T 1824 IN HEALTHY NEWBORN INFANTS

The Influence of the Placental Transfusion

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Since the dyestuff T 1824 is known to be bound to plasma albumin *in vivo* its disappearance from the blood stream during the first hours after injection may be taken as an expression of the transcapillary escape rate of albumin In a previous study (6) this method was used for the examination of infants born of diabetic mothers and a transcapillary escape rate of 20 7 per hour was demonstrated This is a value almost twice as high as that observed in adults (9)

In order to evaluate the significance of this phenomenon we have later examined healthy newborn infants of non-diabetic mothers and in addition we have evaluated the significance of the placental transfusion for the transcapillary escape rate of T 1824 (TER 1824) When late clamping of the umbilical cord is applied about 100 ml of blood is transferred to the infant via the placental transfusion and it was to be expected that this might increase the escape rate (1) Accordingly findings in early and late clamped infants were compared

MATERIAL AND METHOD

The principle used for the determination of the extinction of T 18 4 in plasma has been described in an earlier communication (6) In this study a Gilford spectrophotometer has been used instead of a Zeiss spectrophotometer but the two types were repeatedly compared and found to be of identical stability Furthermore the concentration of T 1824 in plasma was calculated on the basis of a calibration curve showing rectilinear dependence between the concentration of T 1824 in the range 4 54–16 66 μg/ml and its extinction in plasma The coefficient of variation at a given plasma concentration was within the range 0 8–5 6

The procedure used for blood sampling did not differ from that previously described The fall in plasma extinction after injection of T 1824 (0 3mg/kg) was determined on 4–6 blood samples drawn at intervals of 10 minutes throughout 1 hour after injection into the umbilical vein the same vessel being used for blood sampling The regression equation for the fall in extinction of T 1824 served as a basis for the calculation of the transcapillary escape rate of T 1824 using the formula

$$\text{TER 1824 (/hour)} = 100 - \frac{\text{extinction}_{t\ \text{min}}\ 100}{\text{extinction}_{0\ \text{min}}}$$

The correlation coefficient was in all cases significant at the 0 05 level and a correction was made whenever concentrations of plasma proteins in total changed significantly during the examination The actual plasma volume PV (ml/kg) was calculated on the basis of the extinction at zero time The plasma volume at the time of birth was estimated on the basis of the actual plasma volume and the haematocrit value in placental blood since the erythrocyte volume was assumed to be unchanged (9) Haematocrit values (Hct) and the concentration of plasma proteins in total T P (g/100 ml) were determined on all samples as previously described The term val

disorders Epithelial cells may be more likely to manifest such transport defects but technical difficulties in culturing epithelial cells have thus far prevented such studies

SUMMARY

Transport of lysine and glycine was studied in cultured fibroblasts of a patient with Lowe s oculocerebrorenal syndrome Although patients with this syndrome demonstrate renal and intestinal amino acid transport defects no abnormality was found in our study

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Table 1 *Normal newborn infants Transcapillary escape rate of T 1824 (TER 1824) plasma volume (PV) haematocrit (Hct) and concentration of plasma proteins in total (T–P) Mean values ± S D in infants with early (EC) and late (LC) clamping of the umbilical cord*

			At birth			At examination		
Group	No	TER 1824 (/hour)	T–P (g/100 ml)	Hct	PV (ml/kg)	T–P (g/100 ml)	Hct	PV (ml/kg)
EC	5	19 1	5 2	50 2	37 6	5 3	47 7	41 5
		±4 7	±0 7	±6 1	±4 3	±1 0	±6 7	±5 4
LC	7	25 0	5 5	50 1	66 5	5 7	64 1	36 4
		±4 3	±0 5	±3 9	±17 3	±0 4	±3 4	±5 5
p		0 047	n s	n s	0 005	n s	0 003	n s

ues at birth denotes values determined on placental blood

The series comprises 12 infants examined within the first 23 hours after birth in 10 cases within the initial 3 hours Mean birth weight and gestational age were 2 973 g and 38 weeks respectively Pregnancy had been normal in all cases except that twins had been present in 2 cases the amniotic fluid had been discharged early in one case and pre eclampsy had occurred in one case Vacuum extraction was required in 3 cases owing to fetal bradycardia (1 case) secondary abatement of labour (1 case) and pre eclampsia (1 case) Delivery had in all cases been via the vaginal route Manual expression proved necessary in one case the child concerned was born in a markedly transverse presentation Apgar scores were normal in all cases The same applies to the neonatal period apart from two cases in which hyperbilirubinaemia occurred Early clamping which means clamping of the umbilical cord within 15 seconds after birth was used in 5 cases In 7 cases clamping was not done until several minutes after birth

The foetal blood which remained in the placenta after clamping (residual placental blood) was measured according to Redmond s method (8) upon which the placenta was weighed The volume of the residual placental blood is expressed in ml per 100 g placenta

RESULTS

It appears from Table 1 that the plasma volume remained unchanged throughout the period between birth and examination in infants whose umbilical cords were clamped early Contrary to this the estimated plasma volume at birth was high in infants whose cords were clamped late However it was later reduced and at the time of examination had reached a level like that observed in cases of early clamping

The haematocrit values observed at the time of examination were much higher in infants whose cords were clamped late and in contrast to findings in cases of early clamping there was a steep rise from the values observed in placental blood

The concentration of plasma protein in total was identical in all infants no matter whether the umbilical cord had been clamped early or late the latter applies to findings in placental cord blood as well as to findings at the time of examination

The transcapillary escape rate of T 1824 was found to be highest in infants whose cords were clamped late

It finally appears from Fig 1 that the rise

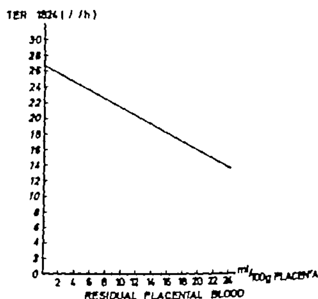


Fig 1 Scattergram expressing the relationship between the transcapillary escape rate of T 1824 (TER 1824) and residual placental blood volume in 12 normal newborn infants

in TER 1824 parallels the fall in the residual placental blood. The coefficient of correlation was -0.587 ($p<0.05$).

DISCUSSION

Concerning the plasma volumes and haematocrit readings the present investigation illustrates the previously (4, 10, 11) described postnatal haemodynamic adaptation in infants whose umbilical cords are clamped late: during the first hours after birth their plasma volumes are reduced. Our study also shows that no essential change in the concentration of plasma proteins in total occurs in this period. Thus the concentration of protein in the fluid which has disappeared into the interstitial space must equal that of plasma.

According to others, TER 1824 has been found to range at 12.8 (2), at 20 (5) and at 13 (3) per hour in the immediate postnatal period. No matter the methods used for clamping the umbilical cord we have found a mean value of 22.6 ± 5.3 per hour. However, we have also demonstrated a higher value following late clamping (25.0 ± 4.3) compared with early clamping (19.1 ± 4.7). The significance of this emerges from a comparison made with early clamped infants of diabetic mothers previously described. TER 1824 was found to range at 20.7 ± 5.1 per hour in these infants and hence the diabetic state of the mothers has hardly any influence *per se* on the disappearance rate.

The cause of the increased disappearance rate of T 1824 in newborn infants compared with adults is unknown. Our study demonstrates that TER 1824 increases parallel with the magnitude of the placental transfusion. This one has been estimated from the volume of the residual placental blood: the smaller the latter, the larger the placental transfusion. Possibly TER 1824 may even reach values met with in adults if extreme early clamping of the umbilical cord is performed.

It may seem remarkable that different values of TER 1824 were found in early and late-clamped infants at a time when their plasma volumes had in fact been adjusted to the same size, just as the concentration of plasma proteins in total were identical in both groups. However, assuming that protein which in consequence of the placental transfusion has disappeared into the interstitial space later on re-enters the intravascular compartment, the high disappearance rate in late-clamped infants may reflect a mechanism by which the concentration of plasma protein is kept constant. In this connexion it should be mentioned that the placental transfusion leads to dilatation and fenestration of the cutaneous capillaries and small blood vessels (7). An anatomical structure therefore exists which may explain the more rapid transcapillary exchange of protein in late-clamped infants compared with early clamped.

SUMMARY

The transcapillary escape rate of T 1824 was measured in 12 normal newborn infants and found to be significantly higher among infants with late clamping of the umbilical cord (25.0 per hour) than in early-clamped infants (19.1 per hour). The magnitude of the placental transfusion was assessed from the volume of the residual placental blood and it was demonstrated that the transcapillary escape rate of T 1824 increases parallel to the placental transfusion. The disappearance rate of the early-clamped infants was almost the same as that previously found in early clamped infants of diabetic mothers.

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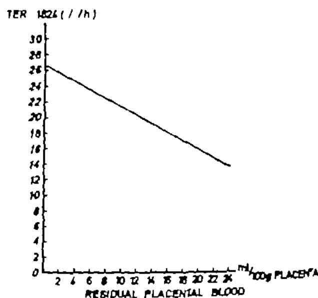


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STAPHYLOCOCCI AND INFECTION IN MATERNITY WARDS

IV Studies on A Partial Rooming in System

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Mortimer et al (6) have demonstrated the importance of direct contact especially by the hands of the personnel for the transmission of staphylococci to newborn babies in maternity wards

It has been argued by Seidemann & Eisenhoff (8) among others that rooming in might reduce the cross infection rate among newborn babies The baby is nursed by the mother in her room in the ward It thus has contact with fewer people than in a conventional maternity ward But Mortimer et al (7) found no decrease in the staphylococcal acquisition rate with rooming in as compared with conventional care

In one of two identical maternity wards at Karolinska sjukhuset a partial rooming in system has ben introduced while the other ward is run on conventional principles The two wards have been studied earlier (1 2 4) before rooming in was introduced

This paper reports staphylococcal colonization and infection rates in the two wards

MATERIAL AND METHODS

In one of the two wards studied ward A a partial rooming in system is practised The child is kept in the ward nursery for the first 2 days after delivery and is attended by the ward nurses On the third day the child is transferred to the mother's room and attended by the mother For morning and evening care the babies are however transferred to the nursery and even after the second day the babies spend 3 to 7 hour[illegible]day in the nursery

In ward B the babies are kept in the ward nursery and are attended by the ward nurses for the whole of their stay in hospital

The babies were *not* hexachlorophene bathed or powdered The staff washed their hands with an ordinary liquid soap followed by an alcohol disinfection of their hands

In both wards most mothers breast feed their babies

The majority of patients were hospitalized for 6–7 days

The study comprises 159 mothers and 160 children 77 mothers and 78 children in ward A and 82 mothers and 82 children in ward B

A swab for bacteriological culture was taken from the nose of the mother on admission to the hospital and from nose and nipples when leaving the hospital Swabs were taken from the babies noses and groins when leaving the hospital All specimens were cultured on blood agar at the bacteriological laboratory of the hospital within a few hours of sampling Sensitivity testing was done on all coagulase positive staphylococci by the quantitative disc diffusion method according to Ericsson et al (3)

All strains with a minimum inhibitory concentration (MIC) <0 1 IU benzyl penicillin/ml <1 µg erythromycin/ml <1 6 µg tetracycline/ml and <4 µg streptomycin/ml were denoted as sensitive to the respective antibiotic Phage typing was done with a standard set of phages on all coagulase positive staphylococci

Nose cultures were also taken from all ward staff every second week Only staff members from whom swabs were obtained on at least three occasions were recorded in the study 32 persons in ward A and 31 in ward B

Mothers and children were observed for clinical signs of infections during their hospital stay When leaving the hospital the mother was given a questionnaire to be answered and returned when the child reached 4 weeks of age In the questionnaire the mother was asked about symptoms of infections in mother child or other members of the family and when these symptoms occurred Missing or incomplete answers were completed by telephone call

Table 1 *Frequency of staphylococcal colonization when leaving the hospital*

	Ward A (rooming in)		Ward B (control)		Total	
	Mothers	*Infants*	*Mothers*	*Infants*	*Mothers*	*Infants*
No *Staph aureus*	52	29	43	43	95	72
Same *Staph aureus* strain as at admission	3	—	15	—	18	—
Staph aureus strain not carried at admission	22	49	24	39	46 (29)	88 (55)
Total	77	78	82	82	159	160

RESULTS

On admission 28 of the mothers (18 %) were nose carriers of Staph aureus

The frequency of staphylococcal colonization in babies and mothers when leaving the hospital is illustrated in Table 1

Thirty six persons among the staff (57%) were never nose carriers of *Staph aureus* Twelve persons (19%) carried the same *Staph aureus* strain throughout the study

There was no significant difference in the colonization rate between the two wards, either in mothers infants or staff

Table 2 gives the phage pattern of the *Staph aureus* isolates from patients and staff in the two wards Phage type 52/52A + dominated among the patients in both wards but not among the staff

Table 3 gives the sensitivity pattern against benzyl penicillin erythromycin tetracycline and streptomycin for the *Staph aureus* isolates from patients and staff No methicillin resistant staphylococci were encountered No significant differences were observed between the two wards as concerns sensitivity pattern of the staphylococci isolated Three of the four multiresistant strains isolated were carried by infected patients

The frequency of infections among the babies and mothers as observed in the hospital and reported in the questionnaire by the mothers is given in Table 4 The numerical difference between the two wards is not statistically significant

The infection rate measured in this way is 27 among the children and 16% among the mothers

As these rates were remarkably high a re evaluation was made of the infections reported in the questionnaires by interviewing the mothers and by studying reports from the child welfare centres that were attended by all children This evaluation clearly showed that infections were grossly over reported especially by nervous and insecure mothers The in

Table 2 *Phage patterns of Staph aureus isolates and number of patients and staff from whom various phage patterns have been isolated*

	Ward A		Ward B	
Phage pattern	Patients	Staff	Patients	Staff
52/52A +	19	3	30	2
Other patterns within group I	4	1	5	2
3A/3C/55/71	2	3	1	0
Other patterns within group II	3	2	3	1
6/42E/47/54/75 +	5	1	6	2
53/83A/85 +	10	0	3	0
Other patterns within group III	4	2	2	1
Other patterns	8	1	10	2
NT	10	7	15	6

Table 3 *Sensitivity patterns of isolates of Staph aureus (139)*

Sensitivity pattern	
Sensitive to benzyl penicillin erythromycin tetracycline and streptomycin	75 (54)
Penicillinase producing sensitive to other antibiotics	60 (43)
Multiresistant	4 (3)

Table 4 *Infection rates in mothers and infants*

Diagnosis	As observed in hospital and reported in questionnaires		After re-evaluation	
	Ward A	Ward B	Ward A	Ward B
Pemphigus	9	7	3	4
Umbilical infection	0	2	0	0
Conjunctivitis	13	5	1	1
Paronychia	3	3	2	–
Mastitis	9	6	2	6
Skin infection in mothers	7	2	1	2
Total	41	25	9	13

fection rate among mothers and children as re evaluated in this way is also given in Table 4 It is in total 7 5 (8 among the babies and 7 among the mothers)

There are no significant differences between the two wards as concerns infection rates whether calculated directly from the questionnaires or as estimated from interviews and reports from child welfare centres

Staphylococcal carriage when leaving the hospital was not significantly more frequent among infected patients than among non infected

DISCUSSION

The two wards studied in the present investigation have been studied earlier (1 2 4) before the partial rooming in system was introduced

It proved very difficult to assess with reasonable accuracy the frequency of clinically manifest staphylococcal infections in mothers and children It is well known that the majority of the infection occur at home after discharge from the hospital (e g ref 2) so that the observations made at hospital are far from adequate The rate of clinical infections as estimated from observations in the hospital and from the questionnaires filled in by the mothers was however remarkably high It was significantly higher than in an earlier investigation from the same wards (2) when the assessment of the infection rate was made in the same way

An investigation among the mothers showed however that they had in many cases grossly over reported infection symptoms in the babies This was especially the case with worried and mentally unstable mothers In fact there was a significantly higher rate of skin rashes and conjunctivitis reported by insecure and anxious mothers than by stable, secure ones (5) When the mothers judgement of the symptoms of their babies seemed doubtful they were therefore interviewed retrospectively in all cases and the reports from the child welfare centres on these babies were studied Thereafter the rate of staphylococcal infections was re estimated The new figures were as remarkably low as the first were high A reasonably true figure for the infection rate can probably only be reached in an investigation in which all mothers are regularly visited by a nurse assessing the infections in mother baby and the rest of the family

However no significant difference was observed between the two wards as concerns the rate of clinically manifest staphylococcal infections in whatever way they were estimated Again therefore no effect of the partial rooming in system in ward A can be proved Nasal carriage when leaving the hospital was as frequent in non infected as in infected cases

We have found no differences in the frequency of staphylococcal carriers among patients or staff in the two wards The partial rooming in system has thus not affected the colonization rate This could be expected since the rooming in system has been far from complete the babies have spent almost a third of the day in the nursery and to a rather large extent have been cared for by the ward nurses In their monograph on Hospital infection Williams et al (9) state that Rooming in can properly serve its purpose only if each mother and her baby are kept together at all times Its value is lost if the babies spend even a short time in a communal nursery

It might be too that to this strict rooming

in system should be added what has been called cohort isolation to gain a full effect on the infection rate so that mothers and babies from one to three days deliveries are segregated in each room and no more are admitted to it until it is empty and cleaned

SUMMARY

Infections and colonization with *Staphylococcus aureus* were studied in 159 mothers and their newborns The patients were observed when in hospital and the mothers were given questionnaires to report symptoms of infection in themselves or their babies during the first weeks after discharge from the hospital The infection rate measured in this way was high 27% among the children and 16% among the mothers Interviews with mothers and reports from child welfare centres that were attended by all children showed however that anxious and insecure mothers over reported infection symptoms Such a re evaluation reduced the infection rates to 8% among the children and 7% among the mothers

No differences were observed in infection rates and in staphylococcal colonization rates between a ward with a partial rooming in system and a similar ward with conventional care A less than total rooming in system thus does not seem to reduce the infection rate

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DETERMINATION OF GLOMERULAR FILTRATION RATE IN THE NEWBORN

Comparison between Results Obtained by the Single Injection Technique without Collection of Urine and the Standard Clearance Technique

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Determination of the glomerular filtration rate by the standard inulin clearance technique in the newborn is difficult because of the low production of urine and hence an increased difficulty for an accurate collection of urine The single injection technique without collection of urine according to the method described by Sapirstein (6) offers an escape from these problems Several authors have published clinical evaluations of this method for determination of glomerular filtration rate in adults and children (3 5 8 9) In these investigations radioactive compounds have been used as indicator substances

The present study was planned as a clinical evaluation with determination of the correlation between the single injection and the standard inulin clearance technique As administration of radioisotopes to the newborn requires special consideration inulin was used as indicator substance

MATERIAL AND METHODS

The mathematical treatment of the single injection technique as described by Sapirstein (6) takes into account continuous equilibration processes between the intravascular and the interstitial pool To study if this balance might be disturbed in the newborn after recent feeding the patients were divided into two groups Sex birth weight and age at investigation of patients in the two groups are shown in Tables 1 and 2 respectively In the first group eleven infants were fasted for 4 hours before the single injection procedure was started (Table 1) In the second group 4 patients were fed within 1 hour before the single injection procedure was started (Table 2) In both groups the single injection clearance without collection of urine was determined first Immediately after this followed determination of inulin clearance according to the standard method The time lapse between the two results in each patient amounts to about 2 hours

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Table 1 *GFR determined by single injection technique (SI) and standard clearance technique (C_{IN}) in 11 infants fasting for more than 4 hrs before determination of SI clearance*

Patient no	Sex	Birth weight (kg)	Age (days)	Glomerular filtration rate (ml/min/1 73 m² body surface) SI	C_{IN}
1	F	3 6	6	31	33
2	F	2 5	4	34	33
3	F	2 6	4	40	39
4	F	3 4	4	29	37
5	F	3 0	5	34	37
6	F	3 0	6	30	36
7	M	3 3	9	52	53
8	M	2 8	5	26	26
9	M	3 7	4	30	35
10	M	2 6	8	30	31
11	M	3.2	14	38	45

in system should be added what has been called cohort isolation to gain a full effect on the infection rate so that mothers and babies from one to three days deliveries are segregated in each room and no more are admitted to it until it is empty and cleaned

SUMMARY

Infections and colonization with *Staphylococcus aureus* were studied in 159 mothers and their newborns The patients were observed when in hospital and the mothers were given questionnaires to report symptoms of infection in themselves or their babies during the first weeks after discharge from the hospital The infection rate measured in this way was high 27% among the children and 16% among the mothers Interviews with mothers and reports from child welfare centres that were attended by all children showed however that anxious and insecure mothers over reported infection symptoms Such a re evaluation reduced the infection rates to 8% among the children and 7% among the mothers

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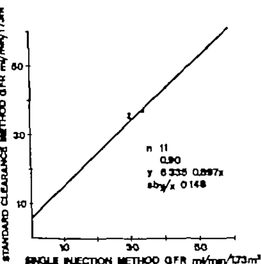
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F 2 Relation single injection method – standard clearance method

RESULTS AND COMMENTS

In Table 1 the results from the 11 infants fasting for 4 hours are shown In this group there is a close correlation between results of the single injection and the standard clearance techniques Correlation coefficient $r = 0.90$ In Fig 2 clearance of inulin is plotted on the ordinate and single injection clearance on the abscissa This gives a regression line $y = 6.335 + 0.897\ x$ The regression coefficient $b_{yx} = 0.897$ and $sb_{yx} = 0.148$ These figures indicate good agreement between the results of the two methods for determination of glomerular filtration rate

As the theoretical model of Sapirstein requires arterial blood sampling inulin was determined in simultaneous blood samples from the vena cava and the aorta in 2 of the 11 infants at 20 40 and 60 minutes after injection of inulin No difference could be found between venous and arterial inulin concentrations This is in agreement with the explanation by Brun (1) that the expected higher concentration of inulin in arterial blood does not show up when glomerular filtration rate is low

It has been pointed out that the single injection technique when using ^{51}Cr EDTA as indicator substance might underestimate the glomerular filtration rate (8) Such a tendency is also found in this investigation

Table 2 shows the results from the 4 infants fed within 1 hour before determination of single injection clearance was started In this group there is a poor agreement between the results of the two methods The values from the single injection procedure amount to about double the values of the standard clearance technique In the mathematical treatment of the two-compartment model Sapirstein puts up the following assumptions (1) After administration the indicator substance is rapidly distributed through a uniform compartment and thereafter penetrates into and distributes homogeneously in a second compartment at a rate proportional to the difference in concentration of the substance between the first and second volume of distribution (2) The substance leaves the first volume of distribution for the bladder at a rate which is proportional to its concentration in the first volume of distribution (3) The bladder and intercompartmental clearances are stable throughout the period of observation The four infants in this second group received breast milk or formula in an amount of 15–20 ml/kg b w 15–45 minutes before the single injection study was started It seems justified to believe that this is enough to start a flux of fluid from the first volume of distribution to the second volume of distribution This would change the intercompartmental clearance during the period of observation and dilute the indicator substance This would result in an increase of the disappearance rate with overestimation of the glomerular filtration rate

The reproducibility of the single injection clearance has not been determined in this study In a study performed in children Donath (2) reports a standard deviation of 5 for repeated determinations in one and the same patient

Table 2 *GFR determined by single injection technique (SI) and standard clearance technique (C_{IN}) in 4 infants fed within 1 hr before determination of SI clearance*

Patient no	Sex	Birth weight (kg)	Age (days)	Glomerular filtration rate (ml/min/1 73 m²) body surface)	
				SI	C_{IN}
12	F	3 4	4	71	26
13	F	3 5	3	93	46
14	F	2 5	5	94	41
15	M	3 4	4	63	43

Single injection clearance

An Argyle® catheter size 3 1/2 Fr was inserted into the umbilical vein and advanced into the vena cava Inulin (Laevasar Gesellschaft 10) was injected in a dose of 100 mg/kg b w with a calibrated glass syringe A sample of the inulin solution was kept for determination of the inulin concentration 0 4 ml of blood was collected before inulin was given and after this every fifth minute for 60 to 85 minutes The samples were collected in small test tubes rinsed with heparin kept in an ice bath until centrifuged Then the plasma was frozen and stored until analysed The chemical analyses of inulin were carried out according to Heyrowsky (4)

The graphic resolution of the experimental curve was performed according to Sapirstein (6) An example is shown in Fig 1 which represents the values from patient No 7 in Table 1 To obtain B the straight part of the curve is projected to intercept on the y axis The steep line with intercept A is constructed by subtraction of the 5 10 15 and 20 minute values of the straight line from corresponding values of the curve The slopes of the two lines γ and γ were determined by dividing the natural logarithm of the ratio of the zero to the 10 minute value on either line by ten *I* in the formula stands for the injected dose of inulin in mg

Clearance of inulin

Standard clearance techniques were used (7) Immediately after the single injection period a continuous infusion of inulin was started through a scalp vein needle (1 mg/kg/min) The equilibration period was 60 to 90 minutes To increase the urine production minimizing the error of collection the patient was given a 5 glucose solution by mouth in an amount corresponding to 1.5–2 of the body weight and thereafter in amounts slightly exceeding the diuresis every 30 minutes For urine collection a double lumen polyethylene catheter was used enabling continuous suction to achieve complete emptying of the bladder Urine was collected in two or three sampling periods for 20 to 30 minutes in each period The urine production varied between 0 2 and 0 9 ml/min Blood (0 4 ml) was collected in the middle of each urine sampling period The chemical analyses of inulin were carried out according to Heyrowsky (4)

In 2 patients Nos 6 and 10 in Table 1 inulin concentrations were determined in both arterial and venous blood at 20 40 and 60 minutes after injection of inulin

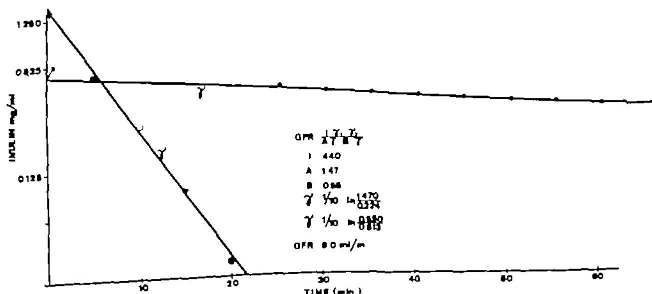


Fig 1 Plasma disappearance curve of inulin (●) and formula used for estimation of glomerular filtration rate Scale is in log line For details see text

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DIAGNOSTIC SIGNIFICANCE OF SERUM OROSOMUCOID LEVEL IN BACTERIAL INFECTIONS DURING NEONATAL PERIOD

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The diagnosis of infections in the neonatal period is inherently difficult since acute infectious disease in the neonate usually lacks specific clinical signs and symptoms Even when acute infection is highly suspected the causative organism cannot always be recovered Several laboratory procedures for detecting neonatal infections have been proposed (1 2 3 4 5 7 8 9) but most of them seem to be of limited value as diagnostic aids

The present report describes the validity of serum orosomucoid estimation as a new diagnostic aid for bacterial infections in the newborn infant

MATERIALS AND METHODS

A total of 342 serum specimens from 90 newborn infants were assayed for orosomucoid concentration

Patients studied were classified into 7 groups according to the condition as shown in Table 1 The first group comprised 10 healthy full term neonates on whom serial estimation of serum orosomucoid levels on cord blood at birth day 7 and 4 weeks of age was carried out None of them had abnormal pre and perinatal history and their clinical courses during the study period were completely uneventful Twenty pre term infants each with an uneventful course during hospital stay served as controls for pre term infants Serum orosomucoid concentrations were serially determined on day 1 2 3 7 2 weeks 3 weeks and 4 weeks after birth Group 3 and 4 comprised infants with established severe bacterial infections Ten infants with bacterial meningitis were classified into the former group and the causative organisms were recovered from their cerebrospinal fluids In the latter group 10 neonates were included on whom the diagnosis of septicemia was established by positive blood culture with apparent clinical manifestation Another 10 neonates with pneumonia belonged to group 5 The diagnosis of pneumonia was made by clinical signs and symptoms with typical chest X ray findings Half of them were considered to have congenital pneumonia since the onset was as early as within 3 days after birth with complicated perinatal history Group 6 included 15 neonates who were thought to have bacterial infections by their clinical manifestations though the causative organisms could not be determined There were 5 infants with necrotizing enterocolitis 5 with neonatal Hirschsprung s disease associated with enterocolitis 3 with urinary tract infections and 2 with panperitonitis due to perforated alimentary tracts The last group consisted of 15 neonates with noninfectious diseases Ten had typical idiopathic respiratory distress syndrome and 5 had Wilson Mikity syndrome No complications with bacterial infection were detected and postmor

Table 1 *Number of cases studied in various groups*

Diagnosis	No of cases	Groups
Normal full term infant	10	1
Normal pre term infant	20	2
Purulent meningitis	10	3
Septicemia	10	4
Pneumonia	10	5
Necrotizing enterocolitis	5	6
Enterocolitis accompanied with Hirschsprung's disease	5	6
Urinary tract infection	3	6
Panperitonitis after intestinal perforation	2	6
IRDS	10	7
Wilson–Mikity syndrome	5	7
Total	90	

SUMMARY

Determination of glomerular filtration rate was performed in fifteen infants by both the single injection technique and the standard inulin clearance technique Eleven infants were fasted for four hours before the study In those patients there was a good accordance between the two methods for determination of glomerular filtration rate Four infants received breast milk or formula within 1 hour before the single injection procedure was started In those infants there was a considerable overestimation of glomerular filtration rate with the single injection technique The error of the method introduced by recent feeding is discussed

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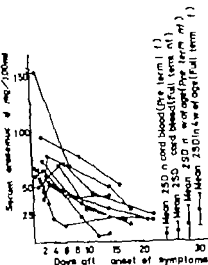


Fig. 3. The elevation of serum orosomucoid in neonatal pneumonia. Congenital pneumonia shows the elevation within a few days after birth. O Congenital pneumonia.

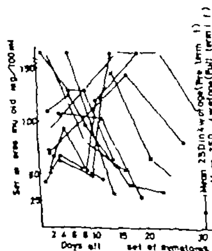


Fig. 4. The elevation of the serum orosomucoid in suspected infectious disease in neonatal period. ○ Necrotizing enterocolitis. ● enterocolitis accompanied with Hirschsprung's disease. urinary tract infection. panperitonitis after intestinal perforation.

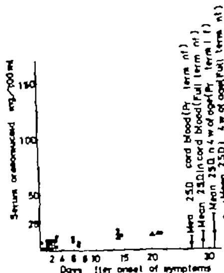


Fig. 5. The serum orosomucoid concentrations in noninfectious diseases in neonates. No significant elevation of orosomucoid is observed in the course of IRDS and Wilson Mikity syndrome. ○ IRDS. △ Wilson Mikity syndrome.

showed significantly high levels of serum orosomucoid from the early stage of the disease. The changing pattern of serum orosomucoid in the course of septicemia was similar to the one seen in cases with established bacterial meningitis as illustrated in Fig. 2.

Fig. 3 indicates the serum orosomucoid levels in neonatal pneumonia. The elevation was not so remarkable as in bacterial meningitis and/or septicemia but it was significant as compared with the control value. Five cases with congenital pneumonia also showed a moderate rise in serum orosomucoid and cord blood specimens already revealed a significant elevation in 2 cases.

Neonates with enterocolitis, urinary tract infections and peritonitis in whom the causative organism could not be recovered also showed a marked elevation in serum orosomucoid concentration as seen in Fig. 4.

Fig. 5 illustrates the values in noninfectious diseases. Infants with IRDS, both survivors and non-survivors, as well as Wilson Mikity syndrome showed an orosomucoid level within normal limits during the whole course.

DISCUSSION

It has been shown that the elevation of serum IgM in the newborn infant might be useful as a diagnostic aid to indicate the presence of infection (1, 2, 7, 8). Serum IgM

Table 2 *Serum orosomucoid levels in normal newborn infants*

	Days after birth									
	0-1d	2	3	4	5	6	7	2w	3	4
Full term infant (10 cases)	8±4 (Cord blood)						27±4			27±7
Pre term infant (20 cases)	6±3	9±4	7±4				10±3	12±4	15±9	15±8

Means ±1 S D (mg/100 ml)

tem examination showed hyaline membranes in all of the 5 patients with IRDS who did not survive

The assay of serum orosomucoid was carried out on capillary blood specimens obtained by heel prick or on cord blood obtained at birth The assay method employed in the present study was single radial immunodiffusion technique utilizing commercially available antibody agar plate (Hoechst West Germany) Each plate was standardized against standard human serum (Hoechst West Germany)

RESULTS

Table 2 shows the results of serially determined orosomucoid levels in the sera from healthy full term and pre term controls Full term as well as pre term infants showed the lowest value on day 1 (8±4 mg/100 ml and 6±3 mg/100 ml) The corresponding value increased gradually reaching the levels of 27±7 mg/100 ml and 15±8 mg/100 ml respectively by 4 weeks of age In the pre term control the concentrations of serum orosomucoid at day 1 7 and 4 weeks after birth were significantly lower compared with those of healthy term infants

Infants with established bacterial meningitis revealed markedly high levels of serum orosomucoid from the early stage of the disease as shown in Fig 1 The value returned to normal range along with the clinical improvement Thus the change in orosomucoid concentration was closely correlated to the clinical course of the disease

Neonates with established septicemia also

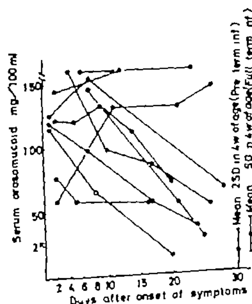


Fig 1 The serum orosomucoid level in established bacterial meningitis Note the markedly high level in the beginning and gradual fall along with the clinical course Normalization is accompanied by the clinical improvement

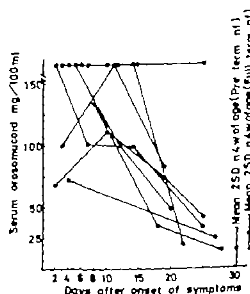


Fig 2 The elevation of the serum orosomucoid in established septicemia The changing pattern is similar to the one in bacterial meningitis

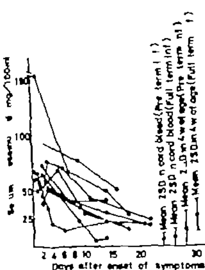


Fig 3 The elevation of serum orosomucoid in neonatal pneumonia Congenital pneumonia shows the elevation within a few days after birth O Congenital pneumonia

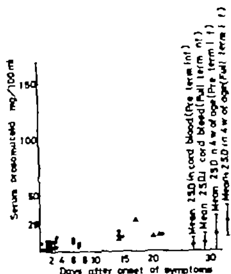


Fig 5 The serum orosomucoid concentrations in noninfectious diseases in neonates No significant elevation of orosomucoid is observed in the course of IRDS and Wilson Mikity syndrome IRDS △ Wilson Mikity syndrome

showed significantly high levels of serum orosomucoid from the early stage of the disease The changing pattern of serum orosomucoid in the course of septicemia was similar to the one seen in cases with established bacterial meningitis as illustrated in Fig 2

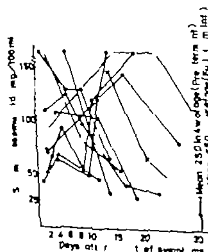


Fig 4 The elevation of the serum orosomucoid in suspected infectious disease in neonatal period o Necrotizing enterocolitis θ enterocolitis accompanied with Hirschsprung's disease urinary tract infection peritonitis after intestinal perforation

Fig 3 indicates the serum orosomucoid levels in neonatal pneumonia The elevation was not so remarkable as in bacterial meningitis and/or septicemia but it was significant as compared with the control value Five cases with congenital pneumonia also showed a moderate rise in serum orosomucoid and cord blood specimens already revealed a significant elevation in 2 cases

Neonates with enterocolitis urinary tract infections and peritonitis in whom the causative organism could not be recovered also showed a marked elevation in serum orosomucoid concentration as seen in Fig 4

Fig 5 illustrates the values in noninfectious diseases Infants with IRDS both survivors and non survivors as well as Wilson Mikity syndrome showed an orosomucoid level within normal limits during the whole course

DISCUSSION

It has been shown that the elevation of serum IgM in the newborn infant might be useful as a diagnostic aid to indicate the presence of infection (1 2 7 8) Serum IgM

however does not always show significant elevation in the early stage of disease (8) Blankenship et al (2) recommended serial estimation of IgM for the detection of infectious diseases in neonates

CRP is hardly detectable in cord sera but even healthy infants often show positive CRP in their sera during the first week of life (5) Positive CRP in the sera may be of value as a diagnostic aid for infection after first week of life since the healthy infants over a week of age normally show the negative CRP (4)

Saxstad et al (9) estimated CRP concentrations more accurately and specifically with immunodiffusion method and found that the incidence of positive CRP varied according to the kind of diseases According to their report CRP appeared in only 1/3 of cases with gastroenteritis but was present in high level in all the cases with pyelonephritis They also demonstrated a good correlation between the change in CRP levels and clinical courses

The concentration of orosomucoid in the cord serum has been thought to be rather high (6) However estimation by the immunodiffusion method revealed that the orosomucoid level in cord sera was very low and that the value gradually increased with age There are no reports so far that the remarkable elevation of serum orosomucoid level is found in the neonatal bacterial infection Our results clearly demonstrated the significant rise of this protein fraction in the severe neonatal infections such as bacterial meningitis septicemia pneumonia enterocolitis urinary tract infections and panperitonitis Serum orosomucoid level was elevated in the very early stage of the disease and returned to the normal range along with the clinical improvement These findings indicate that determination of the serum orosomucoid level is a useful diagnosic aid in bacterial infections in the newborn infant

SUMMARY

The concentration of serum orosomucoid was determined in 90 newborn infants by single radial immunodiffusion method The values were low in both healthy full term and pre term infants In 50 neonates with various bacterial infections the orosomucoid levels showed a marked increase from the early stage of the disease They returned to the normal range with the clinical improvement In infants with non infectious diseases no elevation was detected in any stages of the disease Thus the assay of serum orosomucoid concentration appears to be a useful diagnostic tool for bacterial infections in newborn infants

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A FOLLOW UP STUDY OF CHILDREN WITH ASTHMATOID BRONCHITIS

I *Skin Test Reactions and IgE Antibodies to Common Allergens*

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In children with respiratory tract infections wheezing is a common symptom (8) and it is regarded as an indication that the child runs a high risk of developing asthma The asthma frequency reported among children with previous wheezy bronchitis varies considerably however probably depending on different diagnostic criteria and more or less biased materials (25 34) Although an asthma frequency as low as 5 has occasionally been reported (25) most series have shown a frequency about 20 or higher (5 9 10 36) A follow up investigation of children with asthmatoid bronchitis therefore seemed a suitable means of studying the development of reaginic allergy

In 1967 the discovery of antibodies belonging to a new class of immunoglobulins IgE was reported (18) These antibodies had most of the characteristics known for reaginic antibodies (17) The same year the radioallergosorbent test (RAST) a technique to measure antibodies of the IgE class to particular allergens was described (35) The concentration of IgE antibodies in allergic sera as measured by RAST is significantly correlated with the reaginic activity as measured both *in vivo* (13) and *in vitro* (12)

The aim of the present investigation has been to study the development of reaginic allergy in children with asthmatoid bronchitis by the use of skin prick tests and RAST and to relate the results of these tests to the clinical course of the disease

MATERIAL

The definition of the term asthmatoid bronchitis is the same as was used in an earlier study (11)

Clinical material

The group under study consisted of 81 children 54 boys and 27 girls, of which 53 were first time wheezers when accepted for the study Twenty-eight children had previously had one or more episodes of asthmatoid bronchitis All but one of the 72 children (aged 2–66 months) from a previous study (11) were followed for 27–40 months (mean 32 5 months) A further 10 children 2 11 months old were followed for 15–34 months (mean 18 2 months) from their first attack of asthmatoid bronchitis A history of previous allergic symptoms was obtained in 3 children who were regarded as having had atopic eczema

At the end of the investigation period the children were divided into four groups according to the clinical history The following definitions of the groups were used *Group 1* (*asthma*) At least one attack of apparently exogenously provoked asthma or at least two attacks of wheezing with no apparent connection with a respiratory tract infection *Group 2* (*"asthmatoid bronchitis"*) Wheezing only in connection with respiratory tract infections and with one or more such attacks during the last year *Group 3* (*other allergy*) Atopic eczema or at least one attack of urticaria or at least two attacks of allergic rhinoconjunctivitis without any evidence of asthma *Group 4* (*healthy*) Children with wheezing episodes only in connection with respiratory tract infections and with no such symptoms during the last year

With these definitions 19 children were assigned to group 1 19 children to group 2 8 children to group 3 and 35 children to group 4 Of the 8 children in group 3 two had allergic rhinoconjunctivitis and six

Table 3 *Prick test results matched against the clinical diagnosis*

Prick test result	Patient groups: Group 1 (Asthma) n = 19		Group 2 (Asthmatoid bronchitis) n = 19		Group 3 (Other allergy) n = 18		Group 4 (Healthy) n = 35	
Negative	9		14		3		31	
+	5	53	2	26	2	63	2	11
++	1		1		0		2	
+++ or more	4		2		3		0	

ship between the size of the skin test reactions and the serum concentration of IgE antibodies as measured by RAST is seen in Table 2 The stronger the prick test reactions were the better became the correlation with the RAST results

From the first to the second test occasion the prick test became positive or increased in strength in 31 instances Corresponding IgE antibodies in serum as measured by RAST were found in 13 of these instances in 12 of which an increasing antibody concentration was found An increase of the prick test reaction from negative to weakly positive (+) was accompanied by a positive RAST in the last serum sample in only 3 of 18 cases

Prick tests—clinical diagnosis

The results of the prick tests in the different diagnostic groups may be seen in Table 3 The difference between the results for the healthy group on the one hand and the asthma group or the group with other allergy on the other is statistically significant (χ = 10 89 $p<0\ 001$ and χ = 10 26 $p<0\ 005$ respectively) The skin test results of the group asthmatoid bronchitis did not differ significantly from those of any other group

RAST—clinical diagnosis

Table 4 shows the RAST results for the different diagnostic groups A significantly lower frequency of positive RAST reactions was found in the healthy group than in any of the non healthy groups ($p<0\ 01$) However the majority of the positive reactions in all groups were to egg white and/or cow s milk only

IgE antibodies to animal danders pollens and house dust

A total of 10 children had serum IgE antibodies to animal danders pollens and/or house dust In 5 children the antibodies were present in the first sample (Table 1 No 3 4 5 7 and 8) in the others the antibodies appeared

Table 4 RAST *results matched against the clinical diagnosis*

Figures in parentheses are those obtained when RAST reactions to food allergens are excluded

RAST	Patient groups: Group 1 (Asthma) n = 19			Group 2 (Asthmatoid bronchitis) n = 19			Group 3 (Other allergy) n = 8			Group 4 (Healthy) n = 35		
Negative	9		(15)	9		(16)	3		(6)	29		(34)
Positive 3 10 %	5	53 %	(0)	5	53	(1)	2	63 %	(0)	4	17	(1)
Positive > 10 %	5		(4)	5		(2)	3		(2)	2		(0)

Table 2 *The relationship between the results of skin prick tests and RAST*

Prick test result	RAST result Negative	Positive 3–10 ‰	Positive >10 ‰	positive
Negative	1 122	11	10	2
+	31	3	5	20
+ +	7	2	3	42
+ + + or more	2	2	10	86

urticaria Two of the latter children had one attack each of asthmatoid bronchitis during the last year

Follow up procedure

Physically examination was performed by the author during the attack of wheezing when the child was accepted into the study at a follow up about 3 weeks later and thereafter 3–4 times a year Telephone consultation was also common when the children were ill The follow up visits were almost exclusively conducted during symptom free periods Blood samples were taken at all visits

Skin prick tests were done with 7 allergen extracts (without food extracts) during the first year of study on all 71 children in the original group and with 9 allergen extracts at the end of the investigation period on all but 2 of the 81 children Bronchial provocation tests were performed within 6 months after the last visit

Blood samples

Venous blood samples were taken at the first two visits and with a few exceptions at the end of the investigation period Capillary blood samples were taken at all other visits The mean number of blood samples per child was 9 6 When the samples had clotted the sera were withdrawn and kept frozen at −20 C until analysed However the first three serum samples from the first 71 children were thawed for use in another study (11) and then refrozen

Allergens

Aqueous extracts (1/10 w/v) of danders from horse dog and cat of pollens from birch timothy and dandelion and of egg white and fish were purchased from Vitrum Laboratories Sweden Furthermore a house dust extract (10 000 PNU) from DOME Chemicals Inc New York and commercial skimmed cow's milk were used the latter allergen in RAST only With the exception of the initial skin tests identical batches of the extracts were used at suitable dilutions in all in vivo tests and in RAST

METHODS

Skin tests were performed as prick tests on the volar surface of the forearm The stock solutions (1/10 w/v and 10 000 PNU respectively) were used for the tests and the wheals were measured after 15 minutes A wheal size of 1×1 mm was the smallest called a one plus (+) reaction The wheal obtained with a histamine solution 1 mg/ml was regarded as a three plus (+ + +) reaction and each additional plus indicated a doubling of the wheal size

Bronchial provocation tests were performed by inhalations from a pneumatic air nebulizer (PARI standard 088 Pari Werk) One ml of Coca's solution was nebulized and inhaled as a negative control followed by increasing concentrations of the allergen The reactions were recorded by measuring the peak expiratory flow and by auscultation of the lungs A 15 decrease or more of the peak expiratory flow along with signs and symptoms of bronchial obstruction was regarded as a positive provocation test Most of the children with strongly positive prick test—or RAST reactions were provoked In three of the 22 provocation tests the children did not cooperate in the peak flow examination therefore clinical signs and symptoms became the only diagnostic criteria

RAST (35) was performed as has been described earlier (19) In brief 1 ml of the allergen extracts (1/10 w/v 10 000 PNU and undiluted skimmed milk respectively) was coupled to 100 mg CNBr activated microcrystalline cellulose particles (kindly supplied by Pharmacia Ltd Uppsala) Of this conjugate 0 5 mg in 0 5 ml phosphate buffer was incubated overnight with 50 µl of the serum to be tested at dilution 1/2 to 1/4 After repeated washings 0 1 ml of ^{125}I labelled rabbit anti Fcε (ND) was added The tubes were then incubated overnight and after repeated washings the remaining radioactivity on the particles was measured in a gamma scintillation counter The first and the last serum samples were screened at dilution 1/3 against all 10 allergens In those cases where a positive screen test was obtained all serum samples taken from the patient were tested simultaneously to this allergen However only the first and the last serum samples were tested against the milk allergen Sera from 25 healthy children 6 weeks to 10 years old (mean 2 1 2 years) and with no history of allergy were used as controls for IgE antibodies to the egg and milk allergens The concentration of IgE antibodies was expressed as per mille (‰) of that of a reference birch reaginic serum (19) Results below 3‰ were regarded as negative Positive results were divided into those below and those above 10‰ of the reference serum The unspecific uptake of radioactivity on the cellulose particles was higher in the egg white and cow's milk analyses than in the birch allergen tests The difference in counts obtained when an umbilical cord serum was tested against the food and the birch allergens was therefore subtracted from the counts obtained with patients sera tested against the food allergens prior to comparison with the reference serum

RESULTS

Prick tests—RAST

A total of 65 positive skin tests were found in 24 of the 81 children (Table 1) The relation

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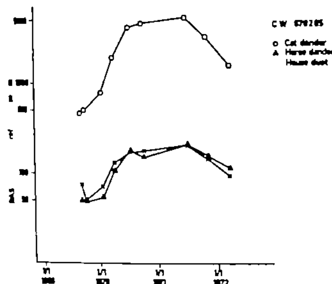


Fig 2 The serum concentration of IgE specific for three different allergens in an asthmatic child not given immunotherapy

improvement was observed Another child (No 4) had to be given rush hyposensitization treatment because of troublesome asthma During the first year of treatment the antibody concentrations were doubled to tripled and IgE antibodies were also detected to two new allergens After one year of treatment the concentrations began to decrease and 1 year later they were below pre treatment levels for all the allergens included in the hyposensitization treatment (Fig 3) This child was also clinically improved during the last year

IgE antibodies to food allergens

IgE antibodies to egg white and/or cow's milk were found by RAST in 26 children (32) and in the first serum samples the concentrations usually were at their highest As the children grew older a marked decrease in antibody concentration was usually seen In most of the patients however only low antibody titres to the food allergens were found Twelve of the 20 children with demonstrable IgE antibodies to egg white had concentrations exceeding 10‰ (Fig 4) Only 3 of these (Table 1 Nos 6 7 and 8) had positive prick test reactions and these reactions were 3+ or more All 3 children had a RAST value exceeding 100‰ and they all had episodes of urticaria in 2 of them definitely caused by egg The third child refused to eat egg Only one other child had a high RAST titre to egg white but

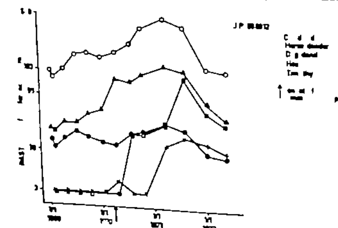


Fig 3 The serum concentration of IgE specific for five different allergens in an asthmatic child given immunotherapy with aqueous extracts of house dust and of danders from horse and cat

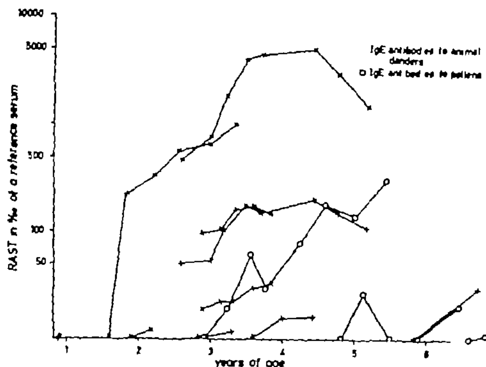


Fig 1 The occurrence of serum IgE antibodies to animal danders and pollens in relation to age (The patient given immuno therapy and patients with a concentration less than 10‰ are not included)

during the follow up period (No 1, 2 9 11 and 25) At the end of the investigation period 4 of the children were considered to have asthma, 3 still asthmatoid bronchitis 1 urticaria and 1 allergic rhinoconjunctivitis One child (No 25) was regarded as healthy but in his last serum sample he had a weak RAST reaction to cat dander

Strong prick test reactions and high RAST titres were usually found only in children with obvious allergy There were two exceptions however At the end of the follow up period one of the children (No 1) still had episodes of asthmatoid bronchitis and no other symptoms of allergy according to the parents Nevertheless he had a very strong prick test reaction and a high RAST titre to the horse dander allergen and at the bronchial provocation test he developed asthma at the allergen dilution 10^{-4} w/v The other child (No 5) with wheezing only in association with respiratory tract infection had a 4+ skin test reaction and demonstrable serum IgE antibodies to the birch pollen extract She had no allergic symptoms at all during the birch pollen season Still she had a positive bronchial provocation test with the birch pollen extract at dilution 10^{-3} w/v

In all the children with manifest reaginic allergy IgE antibodies were detected in serum before their allergy was clinically apparent However in one child with asthma (No 9) IgE antibodies to timothy pollen were found the same summer she had a slight rhinoconjunctivitis This was noted 1 year before she developed asthma

IgE antibodies to animal danders were not detected before 1 1/2 years of age and such antibodies to pollens not before 3 years of age (Fig 1) In all but 2 of the children with IgE antibodies against these allergens an increase of the antibody concentration was seen during the follow up period In all the patients with demonstrable IgE antibodies to pollen allergens a seasonal variation of the antibody concentration was seen The titres usually decreased during the autumn and winter until the next pollen season when they again increased In 1 patient (No 9) IgE antibodies to the birch and dandelion allergens were found only in the summer

In one of the children (No 3) with IgE antibodies to cat and horse dander and to house dust, the concentration of antibodies increased during the first 2 years but thereafter decreased (Fig 2) During this last year a clinical

A seasonal variation of the concentration of reagins to pollen allergens has been reported by several authors using different techniques (3 23 24) Also in the present study a decrease of the concentration of IgE antibodies to pollen allergens was seen during the winter and in one case RAST was positive to two different pollen allergens only in the summer after stimulation of the reagin synthesis during the pollen season When serum tests are used for the diagnosis of seasonal allergy it is therefore important to have a blood sample taken during the weeks following allergen exposure to make full use of the test

The grouping of the children on the basis of the case history only has several limitations The fact that the diagnosis in most cases was settled after less than 3 years follow up may cause a false distribution amongst the different diagnostic groups It may be supposed that some of the children with asthmatoid bronchitis do have asthma or will develop asthma in the near future Most of the children have just reached the age when for example pollen allergies begin to appear The difficulty in differentiating between asthmatoid bronchitis and asthma in wheezing children is well known and in this study 5 children had in fact IgE antibodies to animal danders pollens or house dust when they were included in the study Three of these children developed manifest exogenous asthma and 1 child recurrent urticaria within a year At the end of the investigation period the fifth child only had wheezing when she was infected. She was however skin test positive and had a positive bronchial provocation test to the birch allergen extract One more child finally grouped as asthmatoid bronchitis in fact also had asthma as shown by an increasing RAST titre a strongly positive prick test and bronchial provocation test A probable mechanism behind the recurrent symptoms of asthmatoid bronchitis in some children is that they are exposed to allergens to which they have a low or moderate grade of hypersensitivity Under normal conditions they are free from allergic symptoms but when they become infected with respiratory tract viruses or bacteria the bronchial reactivity will increase (2 26) Exposure to the same concentration of allergens as before may then give rise to symptoms of asthma

Another possible source of error in the classification of the patients may be that some of the children in group 4 are not healthy The absence of apparent wheezing episodes during the last year in no way guarantees that they do not have reaginic asthma or other atopic allergy caused by other allergens than those included in the study

The limited number of allergens used may also contribute to a falsely low correlation when prick test and RAST results are related to the four patient groups This may explain why among the children not regarded as healthy (groups 1–3) 26 of 46 (57%) had negative skin tests and 21 of 46 (46) were RAST negative Among children regarded as healthy no strong prick test reactions were obtained Nor were any high RAST values found except against egg white Thus the results suggest that a strong prick test reaction and/or a high RAST value is highly indicative of reaginic allergy The clinical significance of less strong reactions however must be interpreted with caution

Most children with hypersensitivity to inhaled allergens such as danders and pollens became worse with time and showed increasing titres of IgE antibodies The reverse was seen in isolated cases however A decrease in the concentration of IgE antibodies was accompanied by a clinical improvement both in the child who was spontaneously improved (Fig 2) and in the child given immunotherapy (Fig 3) The benefit of hyposensitization treatment in asthma has been established by several workers (1 6 14 20 22) but the very similar course immunologically and clinically in these 2 children shows the difficulty in the single case of evaluating the effect of treatment

Serum IgE antibodies against food allergens

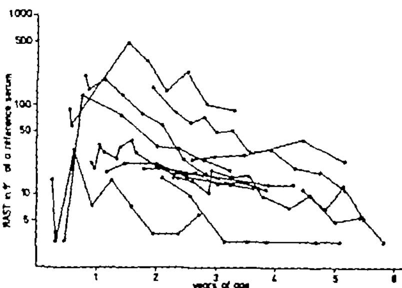


Fig 4 The occurrence of serum IgE antibodies to egg white in relation to age Patients with a concentration of less than 10‰ are not included ● = children with clinical allergy to egg

he was skin test negative and had no history of clinical hypersensitivity to egg

Low concentrations (3–14‰) of serum IgE antibodies to cow's milk were found in 13 children In all patients a higher concentration was seen in the first than in the last serum sample and in 11 of the children milk antibodies were found only in the first sample None of them had a history indicating milk allergy

During the first year of life the most commonly found IgE antibodies were those to food allergens and such antibodies could be found as early as at 3 months of age (Fig 4) Of 46 infants less than 1 year old IgE antibodies to egg white and/or cow's milk were found in 9 and the only other positive RAST reaction in this age group was a weak reaction to house dust in one of the patients with egg allergy (No 8) The 9 infants with and the 37 infants without IgE antibodies to the food allergens in their first serum samples did not show any marked difference in the distribution amongst the groups 1–4 In the 25 control sera IgE antibodies to egg white and/or cow's milk were found in 14 (56%) with titres varying between 3 and 12 9‰

DISCUSSION

Skin prick tests have been commonly used for the detection of skin fixed reagins in allergic patients and good correlations have been reported between the sizes of the wheals and the concentration in serum of IgE antibodies to the particular allergens (30) The prick test technique is especially suitable for use on infants and small children as it implies only a slight discomfort compared with the intradermal test The prick test is however less sensitive than the intradermal test (4) As an attempt to make full use of the sensitivity of the prick test in the present study a wheal of 1 × 1 mm size was regarded as a positive reaction Only a minority of the one plus (+) prick test reactions were accompanied by a positive RAST reaction This may indicate a higher sensitivity of the skin test than of RAST or that reagins primarily are fixed to tissue mast cells and that a certain tissue saturation of IgE antibodies is needed before antibodies appear in the serum The highly improved correlation between the results of the prick tests and RAST when the former were strongly positive is in support of the latter assumption However some of the weak prick test reactions are probably falsely positive and caused by the local trauma of the test The finding that several one plus reactions only appeared at the first test occasion with no reaction at all at the second supports this hypothesis To avoid too many unspecific reactions in routine prick tests it would perhaps be better to limit the minimal size for a positive reaction to a 2 × 2 mm wheal

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were observed at an earlier age than IgE antibodies to animal danders and pollens Antibodies to the food allergens also seemed to be of little clinical importance except for in a few cases while IgE antibodies to animal danders and pollens usually were accompanied by manifest allergy Among the 26 children with demonstrable IgE antibodies to egg white and/or cow s milk only 3 had a history indicating clinical egg allergy with urticaria and no child had symptoms indicating milk allergy Only the patients with clinical egg allergy had a strong prick test reaction and a high RAST value to the egg allergen The frequency of IgE antibodies at low titres to egg and/or cow s milk was higher among the healthy controls than among the children in the follow up study The results indicate that such antibodies at low titres have no apparent relation to atopic disease

A decrease in the concentration of IgE antibodies to egg white and cow s milk was seen with increasing age in almost all cases With different techniques antibodies to milk proteins of other immunoglobulin classes than IgE have commonly been found in childhood and the occurrence of such antibodies decreases with age (28) Clinical hypersensitivity to egg is often seen in infants with eczema but with increasing age the egg allergy often becomes less pronounced (16) When egg is introduced into the diet at the end of the first year of life it seems to cause less allergic reactions than when introduced earlier (15) Thus, a maturation process appears to influence the liability of the child to produce antibodies to food allergens and in some cases also symptoms of food hypersensitivity As food proteins may be absorbed from the intestinal lumen at any age (27 33) a decreased absorption of unaltered food proteins is hardly the sole explanation of the decreasing antibody production It has been suggested that the maturation process is related to the development of antibodies of the IgA class which normally coat the mucous membranes (21 32) In support of this hypothesis are the findings that precipitating antibodies and high titres of haemagglutinating antibodies to milk proteins are found in increased frequency among patients with IgA deficiency (7) and also in patients with gastrointestinal disorders where the normal barrier function of the intestinal mucosa is damaged (29 31) Protein antigens in the food may then be absorbed at such a concentration and in such a state that antibody formation is induced

SUMMARY

Eighty one children, 2–66 months old, with asthmatoid bronchitis were followed for 15–40 months (mean 31 months) The children were physically examined 3–4 times a year Blood samples were taken on these occasions Skin prick tests were done at the beginning and at the end of the investigation period Antibodies of the IgE class as measured by RAST were found against animal danders pollens or house dust in 5 children at the first examination and during the follow up period in another 5 children Strongly positive prick test reactions were accompanied by a positive RAST in 86% smaller wheals less frequently IgE antibodies to egg white or cow s milk were found in 26 children, in many cases during the first year of life and occasionally as early as at the age of 3 months As the children grew older the titres decreased and the antibodies seemed to be of clinical importance only in a few cases with high titres to the egg white extract In contrast, IgE antibodies to animal danders were not detected until 1 1/2 years of age and those to pollen not until 3 years of age These antibodies showed increasing titres with time and they seemed to be of clinical importance in all but a few cases with very low titres

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TUFTSIN DEFICIENCY SYNDROME

A Report of Two New Cases

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A few years ago we reported the existence of a specific leucophilic γ globulin fraction (leucokinin) that coats the circulating blood polymorphonuclear (PMN) neurtophil (8) Leucokinin which is of the γG class was found to stimulate the phagocytic activity of the neutrophil to a considerable extent (9) We have since shown that this biological activity resides fully in a small peptide tuftsin which is bound to the carrier leucokinin molecule The latter transports tuftsin from its site of activation the spleen to the site of its biological activity the receptors on the outer membrane of the PMN phagocyte (12) Tuftsin has been isolated and its structure determined as threonyl lysyl prolyl arginine (15)

Tuftsin deficiency has been observed thus far in two families (6) It has also been found lacking in splenectomized human subjects as well as in experimentally splenectomized dogs (12 14)

In this communication we report the occurrence of congenital familial tuftsin deficiency in another five subjects belonging to two unrelated families

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CASE REPORTS

Case 1 D I is a 3 year-old white girl who at the age of 4 days had numerous skin infections with *Staphylococcus aureus* mostly in the form of large boils involving the face trunk and lower extremities Since then the patient has been having one or more such boils almost continuously and in various parts of her body surface At the age of 18 months she developed bilateral infection of both the upper and lower eyelids resulting in almost complete loss of her eyelashes There was no response to various antibiotics As a result her physician has since resorted to a protracted regime of biweekly immunization with a commercially prepared Staphylococcus vaccine There has been no obvious improvement after 6 months of immunization She continues to have boils particularly in the axillae chest area and trunk She was brought to our attention shortly before the start of the vaccination The parents and fourteen siblings gave no history of recurrent infections

Physical examination revealed many scars throughout her body which mark past sites of incised boils There were also several healing boils on her trunk and extremities Her eyelids were actively and diffusely inflamed There was no enlargement of the spleen liver or lymph nodes Her tonsils were normal

Blood examination showed hemoglobin 11 g/100 ml hematocrit 34% white blood cells 5 650 per mm neutrophils 49 lymphocytes 49 monocytes 2 platelets were within normal limits These are typical of several determinations made at various intervals Routine blood and urine analyses as well as chest roentgenograms were normal Tuberculin skin test was negative γ Globulins in mg per ml of serum showed γG 10 0 γA 0 0 and γM 0 4 Fractionation of her γ globulin on phosphocellulose column yielded a normal pattern in all four peaks (12) C3 assays

(in Dr Fred S Rosen's laboratory) yielded quantitative values of 135 mg and functional values of 12 663 units per 100 ml of serum both within high normal levels (5) Tuftsin activity in her serum was absent Both parents and eleven siblings were tested Three siblings were not available No deaths were reported

Case 2 W M is a 6 year old Caucasian boy who was in good health until the age of 18 months Since then he has had numerous hospital admissions for multiple skin abscesses recurrent bouts of otitis media two episodes of staphylococcus pneumonia with pyopneumothorax and several episodes of bronchiolitis About 6 months ago he was admitted to the Boston Floating Hospital with fever 39 C vomiting severe abdominal pain and distention

Both parents and three living siblings gave no history of recurrent infection Two siblings died of undetermined causes at the age of a few weeks

The significant findings during physical examination were fever tender liver 7 cm below the right costal margin spleen 2 cm below the left margin and shotty cervical adenopathy

Laboratory data on admission were hemoglobin 8 g/100 ml hematocrit 27 white blood cells 15 700 per mm³ neutrophils 79% lymphocytes 12% bands 4% monocytes 4 and eosinophils 1% Urine culture yielded *Staphylococcus aureus* 30 000 colonies per ml Liver scan showed large defects Four blood cultures revealed *Staphylococcus aureus* γ Globulin in mg per ml showed γG 18 5 γA 2 9 and γM 1 4 Tuberculin coccidioidin and candida skin tests were negative Lymphocyte stimulation by phytohemagglutinin was normal C_3 and C_4 measured 504 and 195 mg/100 ml respectively Reduction of nitroblue tetrazolium (3) by peripheral leucocytes gave high normal values

Intravenous cephalosporin 400 mg per kilogram per day was instituted upon admission His temperature went down to normal in 3 days Blood cultures became negative and white cell count fell to 7 000 per mm³ with normal differential counts However his liver size remained undiminished Forty days after admission the diagnosis of multiple liver abscesses was confirmed by laparotomy and drainage of the pus which grew *Staphylococcus aureus* The patient was maintained on intravenous cephalosporin for a total of 10 weeks after which it was discontinued His temperature rose to 39 4 C 3 days later necessitating a return to intravenous cephalosporin Two other attempts at drainage a month apart again yielded pus with *Staphylococcus aureus* Therapy was continued for another 10 weeks

Studies of serum tuftsin activity were begun 7 months after admission All assays were consistent with the diagnosis of tuftsin deficiency syndrome Not only was there no detectable tuftsin activity but also there was inhibitory activity towards tuftsin obtained from normal γ globulin The patient was immediately put on intramuscular γ globulin therapy 3 0 g a week for 5 weeks Eight days after the start of γ globulin therapy and 9 weeks after the third laparotomy a fourth exploration was made for possible liver resection There were no detectable abscess formations and cultures of liver aspirates were negative Cephalosporin was discontinued 18 days after the institution of γ globulin therapy At this time he remained afebrile with a white blood cell count of 5 500 per mm neutrophils 63% lymphocytes 24% bands 6 monocytes 5 eosinophils 1 and basophils 1% Blood and urine cultures were negative For the first time he remained afebrile for 2 weeks after cessation of cephalosporin therapy This improvement coincided with the institution of γ globulin therapy 32 days earlier

MATERIALS AND METHODS

The materials and methods used in the preparation of samples and assay for tuftsin activity were described in detail elsewhere (6 12) Essentially γ globulin from fresh serum is prepared and subjected to strictly limited trypsin digestion The liberated peptide tuftsin is extracted with ethanol evaporated and dissolved in Krebs-Ringer phosphate buffer An aliquot is assayed for tuftsin activity along with normal γ globulin controls All assays are run in duplicates Quantitative assays of C_3 and C_4 as well as γG γA and γM were made by immunodiffusion

RESULTS

The phagocytic index and the corresponding phagocytic stimulation are shown in Table 1 Phagocytic stimulation is the phagocytic index of the test sample minus the phagocytic index of the reagent control It can be seen in Table 1 that patient D I her mother and only one brother out of eleven siblings tested showed no significant tuftsin stimulation Patient W M and his father were deficient Only the mother who had normal tuftsin levels was available for study All values are representative of three measurements made at different times

The possibility that the patients may have a mutant tuftsin peptide that inhibits normal tuftsin activity as was shown in previous cases (6) was investigated Indeed we found that trypsin digest of the γ globulin of both patients exhibits strong antagonism to natural tuftsin obtained from a digest of normal γ globulin under identical conditions Table 2 illustrates the lack of stimulation that results when a tuftsin preparation from the patients is mixed with that from a normal subject in a molar ratio of 2 1 respectively

DISCUSSION

Several syndromes have recently been described relating to impaired phagocytosis (a) deficiencies of bactericidal activity (2 7 10 16) (b) deficiencies of serum complement factors such as C3 C5 and (c) composite deficiency in animals involving C5 C6 and C7 (1 11 18) All these involve the opsonization of the target particle By contrast tuftsin deficiency represents an impairment of the engulfing activity of the blood neutrophil Tuftsin acts directly on the phagocyte and has no effect on the bacterial particles

Tuftsin activity is present only in the leucophilic fraction of γ globulin leucokinin which is 4% of the total γ globulin This leucokinin molecule which is either synthesized or activated by the spleen binds at the proper receptor site on the outer surface of the PMN cell membrane The enzyme leucokininase on the outer surface of the cell membrane cleaves off the tetrapeptide to make it available to the cell (6 13 15)

The use of γ globulin injections as a natural supply of tuftsin is a reasonable substitute

Table 1 *Tuftsin activity in the serum of two families having patients with tuftsin deficiency*

Subject	Age years	Phagocytic index	Phagocytic stimulation (Δ index)
Family of D I			
D I (Case 1)	3	20	0
P I (mother)	42	20	0
P I (father)	47	43	23
C I (brother)	18	42	[illegible]
L I (brother)	4	37	17
A I (brother)	8	39	19
R I (brother)	12	38	18
J I (brother)	14	38	18
S I (brother)	16	44	24
P I (brother)	21	41	21
L I (sister)	2	39	19
M I (sister)	6	38	18
F I (sister)	10	40	20
T I (sister)	11	45	25
Family of W M			
W M (Case 2)	6	20	0
L M (father)	48	20	0
B M (mother)	40	39	19
Normal values	—	47 ± 6	22 ± 6
Reagent control	—		

Table 2 *The inhibition of phagocytic stimulation of normal tuftsin by a preparation of tuftsin from serum of the patients with tuftsin deficiency syndrome*

Case	Diagnosis	Phagocytic index	Phagocytic stimulation (Δ index)
A C	Normal subject	47	27
D I (Case 1)	Tuftsin deficiency	20	0
W M (Case 2)	Tuftsin deficiency	20	0
Mixture D I / A C 2 1	—	19	0
Mixture W M / A C 2 1	—	18	0
Reagent control	—	20	0

for the tetrapeptide tuftsin In our previous cases there was a dramatic response to γ globulin in injections of 0 83–1 65 g (6) Because of that experience Case 2 (W M) was finally treated with γ globulin Considering the history and state of the patient it was felt wise not to wait until another infection set in Thus the efficacy of γ globulin administration in this instance could not be adequately assessed at this time

Case 1 (D I) is currently under observation following a prolonged vaccine therapy She has actually shown no beneficial effect However her physician chose to observe her for another year before attempting γ globulin therapy

Other than the fact that this syndrome is familial it is difficult to evaluate its genetic characteristics An insufficient number of cases have been studied with only two generations available for testing We have found the deficiency to be transmitted through one parent The other parent had normal values It is also clear that the symptomatology is quite variable (6) as is obvious also in this report Several subjects who clearly had laboratory evidence of tuftsin defect did not show any symptoms Other host factors must therefore play a role There are cases with C5 defects

who exhibit no symptoms (11) Defects in bilirubin metabolism vary from severe kinec terus to absence of brain damage (4) About 80% of cases with biochemical characteristics of gout are symptomless (17) Tuftsin deficien cy falls in this category of diseases originat ing in a deficiency of a biologically functional factor

SUMMARY

Two families with deficiency in the phago cytosis stimulating peptide tuftsin are re ported Both cases gave a history of recur rent infections In the first case (D I) all infection involved only external surfaces The second patient (W M) showed involvement of internal organs with septicimia pneumonia and liver abcess in addition to skin lesions In both cases *Staphylococcus aureus* was the infecting organism

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LACTOSE MALABSORPTION IN ETHIOPIAN CHILDREN

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Interest in the incidence of lactose malabsorption and its possible etiology have attracted global attention following reports of widespread incidence of this disorder amongst asymptomatic population groups predominantly from the under developed parts of the world This is attested by the plethora of literature during the past decade on the subject Incidence studies in children are few but seem to indicate that lactose malabsorption is less frequent before weaning and develops progressively with age (9 17 29)

The geographic ethnic and racial differences observed have been explained in theories of genetic and/or adaptive origin (2 3 4 8 14 26 32 34 35) Doubts have been expressed as to whether it is at all desirable to use milk as a source of supplementary food to children in underdeveloped countries or for treatment of protein-calorie malnutrition (11 18 25)

The subject of lactose malabsorption has recently been excellently reviewed by the Protein Advisory Group (30) The review attempts to clarify terminology (which we have followed) summarizes studies from throughout the world and concludes with an assessment of the nutritional implications

As milk is considered a virtually complete food of great value to children of underdeveloped countries it is essential for each country to find out the incidence of lactose malabsorption Data from other parts of Africa (10 20 23 28) point to a very high prevalence even in children As important would be an attempt to evaluate the implications in order to formulate a realistic food and nutrition policy

This paper reports on a study amongst Ethiopian children living in various parts of the country and also of a "control" group of Scandinavian children said to have a very low incidence of lactose malabsorption The study also attempted to test the implications of the high incidence found through a milk feeding trial amongst a defined group of school children

Most of the data on incidence studies have been obtained from lactose tolerance tests which are generally agreed to be fairly good diagnostic tests for lactose malabsorption However there is lack of uniformity in performing these tests particularly with regard to the number and timing of blood samples for glucose determination Although obviously unsuited to large studies traditional methods involved taking at least 5 or 6 blood samples over two hours and were justified to avoid misleading results In the wake of carrying out an incidence survey we set out to re-examine the timing and number of blood samples for glucose determination with a view to look for an abbreviated but equally reliable test for lactose tolerance

Table 1 *Mean blood glucose value at various sampling time after an oral load of lactose 2 g/kg I to 10 year old children from Addis Ababa*

No of cases	Mean blood glucose (mg/100 ml)						
	0	20	30	40	60	90	120
Ethiopian							
17	66 1	—	80 9	—	73 2	69 8	67 3
11	70 6	—	76 5	78 2	77 8	—	—
10	71 2	—	—	86 4	85 5	—	—
32	61 8	70 3	—	68 2	—	—	—
29	76 3	—	—	89 4	—	—	—
Caucasian							
17	63 4	99 2	—	94 6	—	—	—
11	76 3	—	—	101 3	—	—	—

MATERIAL AND METHODS

A *Lactose tolerance test*

One hundred and eighty three Ethiopian children of various ethnic origins and 28 Caucasian children resident in Ethiopia were included in the study The material was collected in the capital Addis Ababa and 4 out of 14 provinces namely Eritrea Harrar Illubabor and Sidamo The persons tested belonged to at least 10 ethnic groups and were mainly living in fairly urbanized centres In Addis Ababa 83 out of 99 children tested were hospitalized and in Eritrea 14 out of 16 All the other children were ambulatory cases tested in the sitting position The sex age weight weight level expressed as percentage of the Boston Standard (50th percentile) (19) parental tribe and primary diagnosis were registered on all children Only children who had no clinical history of diarrhoea and/or vomiting three days prior to the test were accepted In addition children with kwashiorkor were also excluded

After an over night fast or in the youngest group 4 hours fasting 2 g of lactose per kg body weight (maximum 50 g) was given The lactose was dissolved in water to make a 20 solution and administered in a glass or in some cases by naso gastric tube The lactose was found to be free of monosaccharides by checking with chromatography

Capillary samples for glucose determination were obtained from fingerpricks before the lactose load and at various subsequent intervals (Table 1) The Addis Ababa material was used to study the sampling time

The true glucose value was determined by the glucose oxidase method (16) soon after the test was completed in the Addis Ababa material In the studies from the provinces samples were immediately precipitated with perchloric acid and blood glucose determined within 24 hours on return to Addis Ababa Known control samples so treated and kept for as long as 2 days had shown no significant change of glucose level

Lactose malabsorption was diagnosed when blood glucose failed to rise to 20 mg/100 ml above the fasting level on any subsequent sample

B *Milk intolerance*

The practical implication of lactose malabsorption was tested in a pilot milk distribution trial in a governmental school in Addis Ababa Full details of the study will be reported elsewhere Among 460 students in the age group 6–10 years 100 were randomly selected for the study There were 10 girls and 10 boys in each of the five age groups Only very few were used to take milk or milk product daily

All the children were interviewed at noon about the presence of gastro intestinal symptoms abdominal pain nausea loose stools vomiting bloating and buorborygmi Interviews were conducted during the week before one and four weeks after the start of the milk distribution Around 8 a m all the 460 children were supervised during the drinking of 250 ml of pasteurized whole milk This amount was administered five days a week for a total of 4 weeks

RESULTS

A *Lactose tolerance test*

Sampling time and peak rise The mean glucose levels in the groups tested in Addis Ababa and sampled at various times after the lactose load are given in Table 1 In the first group of 17 the 6 absorbers had the peak rise at 30 min (5 cases) and 60 min (one case) In the second group of 11 the only absorber had the peak at 40 min In the third group of 10 the 3 absorbers all had the peak at 40 minutes In the Caucasian material 9 of 14 absorbers had their peak at 40 min

Amongst the 49 children tested at 0 20 and

40 min 23 had a peak of 20 mg/100 ml or above at 20 or 40 min Only two would have been interpreted falsely as malabsorbers if the 20 min sample was not taken This means that taking the additional 20 min sample has uncovered 4 percent absorbers

Frequency of lactose malabsorption As seen in Table 2 the mean frequency of lactose malabsorption among the Ethiopians is 80 The Caucasian group had a frequency of 25%

The clinical signs and symptoms during or a few hours after the test period were followed in the hospitalized patients Symptoms were absent in all but a few and when present were manifested by abdominal cramps and loose stools

The Ethiopian material was further analysed according to sex age weight level and ethnic groups There was no difference between boys and girls in number of responders The peak values at any sampling time were used in correlation with age groups The frequency of lactose malabsorption was found to increase with age (Table 2)

The weights of the patients varied from 70 to 110 of the Boston Standard (19) and analysis of weight level against glucose response revealed no correlation

The origin of the Ethiopians were mainly Amhara and Tigre However there were around 10 cases each where both parents were respectively Galla Anuak Nuer Kotu Adhere or Somali It was not possible to detect any difference in the incidence of lactose malabsorption between any of the Ethnic groups in this material

B *Milk intolerance*

The relative frequency of abdominal symptoms during the baseline week was 3 9 / The first week after the beginning of milk consumption the symptoms at school more than doubled (10 6%) but came close to baseline during the fourth week (4 7) The most frequent symptom registered was abdominal pain which was [illegible] in twice the number of

Table 2 *Percentage of lactose malabsorption according to age*

Age (y)	No of cases	Per cent with rise less than 20 mg/100 ml
Ethiopian		
Under 1 y	26	61 5
1-1 ½ y	17	64 7
2-6 y	82	81 7
7-13 y	58	89 7
Total	183	79 8
Caucasian		
2-13 y	28	25 0

school children during week one Among the children who were suspected of having abdominal symptoms caused by the milk consumption six were tested with lactose tolerance test All had flat curves as had six non symptomatic age matched controls

DISCUSSION

The high incidence of lactose malabsorption diagnosed with per oral lactose tolerance test among Ethiopian children (80%) is in agreement with reports in sub-Saharan Africa as is the progressive incidence with age of lactose malabsorption Our incidence of over 60 in the under 1 year age group (all in patients) contrasts with the 15 and 30 reported by Cook (9) and Kretchmer et al (23) and the 0% claimed by Olatunbosun & Adadevoh (29) The absence of ethnic differences in incidence in this study should not be taken seriously because most of the subjects came from urban settings and very few from cattle herders and other traditional milk consuming groups

Our simple methodological study of lactose tolerance test with various time intervals indicates that 0 and 40 min sampling times give as reliable results as do the more complex traditional test involving more samples This finding agrees with McMichael (27) who also concluded that an abbreviated test taking only two samples (0 30 min) was sufficient

An unexpected finding was the high in

Table 1 *Mean blood glucose value at various sampling time after an oral load of lactose 2 g/kg 1 to 10 year old children from Addis Ababa*

No of cases	Mean blood glucose (mg/100 ml)						
	0	20	30	40	60	90	120
Ethiopian							
17	66.1	—	80.9	—	73.2	69.8	67.3
11	70.6	—	76.5	78.2	77.8	—	—
10	71.2	—	—	86.4	85.5	—	—
32	61.8	70.3	—	68.2	—	—	—
29	76.3	—	—	89.4	—	—	—
Caucasian							
17	63.4	99.2	—	94.6	—	—	—
11	76.3	—	—	101.3	—	—	—

MATERIAL AND METHODS

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All the children were interviewed at noon about the presence of gastro-intestinal symptoms: abdominal pain, nausea, loose stools, vomiting, bloating and buorborygmi. Interviews were conducted during the week before, one and four weeks after the start of the milk distribution. Around 8 a.m. all the 460 children were supervised during the drinking of 250 ml of pasteurized whole milk. This amount was administered five days a week for a total of 4 weeks.

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Amongst the 49 children tested at 0, 20 and

amongst Ethiopian children was found to be 80% being 61 5% in the under 1 year age group and 89 7 in the older children (7–13 years) An unexpected finding was the high incidence of 25 amongst Caucasian children (mainly Scandinavians) Altered intestinal bacterial flora and/or repeated gastrointestinal infections and prolonged reduced intake of milk are offered as possible factors for the high incidence

Milk consumption in normal quantities (250 ml) by a school population of malabsorbers gave rise to "abdominal symptoms in some but this rapidly abated by 4 weeks to reach the preconsumption level This preliminary observation if confirmed should encourage the use of milk as a valuable source of supplementary food in communities where protein calorie malnutrition is widespread

ACKNOWLEDGMENT

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cidence of lactose malabsorption amongst Caucasian children (mainly Scandinavians) residing in Ethiopia Studies in Scandinavia give an incidence varying from 1% to 5% (12) The Scandinavian children in this study were all in excellent nutritional state and healthy and many have been in Ethiopia for a few years However their milk intake since coming to Ethiopia though not quantitated is known to have decreased and they are subject to a higher incidence of gastro enteritis than in their homeland The increase in incidence would have to be accounted for by acquired environmental factors

Keusch et al (21) and Varavithya et al (37) have reported a significantly higher incidence of lactose malabsorbtion amongst children with diarrhoea and/or malnutrition than in normal controls and also a persistence of mal absorption for as long as 5 months Acquired lactose malabsorption is also well documented in other disease states (1 6 15 36)

Impaired small intestinal fuction in asymptomatic apparently healthy native born individuals from many developing countries is now well established as also is the development of impaired intestinal function in expatriates residing in these countries (This subject was extensively reviewed in a symposium on Mal absorption and Nutrition held in Washington in April 1971 and reported in the October and November issues of the American Journal of Clinical Nutrition 1972) In both instances the disorder seems reversible on emigration to a Western country implying that the cause may be related to repeated exposure to an environmental agent or agents present in underdeveloped countries (24)

A study in Nigeria of endemic intestinal disease occurring in asymptomatic, healthy looking subjects concluded that hypoalbuminemia presumably a consequence of poor nutrition is an important factor capable of producing a biochemically and histologically defined malabsorptive state (13)

It is usually assumed that in man dietary lactose does not influence the lactase activity of the intestinal mucosa (22, 31) although contradictory evidence is available in experimental animals (5 7) The combined factors of prolonged lack of substrate stimulation and gastrointestinal infection may contribute to lactose malabsorption In attempting to explain the different age of onset of malabsorption in Finns and Thais Sahi et al (33) have also advanced dietary habits as possible factors in addition to a central genetic basis

It is therefore worthwhile to investigate the possibility of altered intestinal bacterial flora and/or repeated gastrointestinal infections, suboptimal nutritional status, and prolonged reduced intake of milk as contributory factors in lactose malabsorption in asymptomatic, normal children Until such possibilities are properly ruled out, it will be wise to interprete the high figures from countries with poor living conditions with caution

Milk consumption in normal quantities (250 ml) by a school population of malabsorbers (90%) gave rise to abdominal symptoms in some but this rapidly abated by four weeks to reach the preconsumption level Thus even in a population with a low consumption of dairy products and a high incidence of lactose malabsorption the introduction of 250 ml of milk daily in the diet will cause only transient problems This observation is of great importance as the addition of even small quantities of milk and milk products will add substantially to the quality and amount of protein intake of children in communities where protein calorie malnutrition is widespread

SUMMARY

Oral lactose tolerance test was performed on 183 Ethiopian and 28 Caucasian children resident in Ethiopia A study of various sampling time intervals during the lactose tolerance test indicated that the fasting and 40 min samples were sufficient and the most suited for large incidence studies

The incidence of lactose malabsorption

Acta Pædiat Scand 62 655–658 1973

EFFECT OF FASTING AND OF A PROTEIN FREE DIET ON AMINO ACID RATIOS IN PLASMA AND ERYTHROCYTES IN RATS

MAHIN AZAR NAZNIK TER SARKISSIAN MOHSEN BAVENDI
HABIB HEDAYAT[1] and GONZALO DONOSO[2]

From the Food and Nutrition Institute of Iran Teheran

The ratio of non essential (NE) to essential (E) amino acids as determined by a simple paper chromatography procedure has been proposed by Whitehead as a criterion for assessing protein-calorie malnutrition (PCM) in infants and preschool children (1) Although some workers have found this ratio to be valid especially in patients suffering from the kwashiorkor type of PCM (2) others have reported it to be too strongly affected by the immediate intake to be a reliable index of nutritional status (3) Recently the erythrocyte/plasma amino acid distribution ratio has also been found to be altered in PCM (4)

As protein-calorie malnutrition is the result of variable combinations of calorie deprivation and protein deficiency it was thought of interest to study the effect of fasting and of a non protein diet on the plasma and erythrocyte amino acids in rats

Our results show that very different changes are induced in the NE/E amino acid ratios the amino acid concentration and their relative erythrocyte/plasma distribution by fasting and by a non protein diet

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[1] Director
[2] WHO Medical Nutritionist

Stock diet

	(grams)
1 Whole wheat flour	500
2 Wheat	500
3 Barley	500
4 Quaker oats	500
5 Corn	500
6 Sesame	500
7 Milk powder	1 155
8 Vitamin mixture	40
9 Salt mixture	150
10 Corn oil	100

Non-protein diet

1 Maize starch	1 000
2 Glucose	2 000
3 Vegetable fat	500
4 Salt mixture	200
5 Vitamin mixture	50
6 Corn oil	250

METHODS

Twenty six albino Wistar rats approximately 6 months of age were distributed in the following experimental groups

Fasting (F) 10 rats that had been receiving stock diet were fasted for 72 hours

Non protein (NP) 8 animals on stock diet were fed on a non protein diet for 72 hours

Stock diet eight animals on stock diet

The animals were sacrificed and amino acid ratios and concentrations were determined in plasma and in red cell hemolysates by paper chromatography using the methods of Whitehead (1) and Bjornesjo (5)

A standard of glycine for spot 1 (glycine glutamine and serine) of alanine for spot 3 (alanine) of valine for spot 5 (aline methionine) and of leucine for spot 7 (leucine isoleucine) were used to determine the concentration of the amino acids in plasma and erythrocytes from the eluted spots

RESULTS

Table 1 shows the ratios of NE/E amino acids both in plasma and erythrocytes Fasting and non protein diet induce increments in the ra

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	250

Table 1 *Effect of fasting and a non protein diet on the NE/E amino acid ratios in plasma and erythrocytes (mean ± S E M)*

Group	*n*	Gly + Glu + Ser Leu + Isol + Val Plasma		Erythrocytes	
Fasting (F)	8	2 25±0 21		1 70±0 07	
Non protein (NP)	8	1 94±0 15		2 78±0 08	
Stock (S)	10	1 07±0 02		0 84±0 04	
		t	*P*	*t*	*P*
S vs F		5 7	<0 001	15 0	<0 001
S vs NP		5 7	<0 001	28 0	<0 001
F vs NP		2 1	<0 05	9 2	<0 001

tios which acquire high levels of significance especially in the erythrocytes

Table 2 indicates the amounts of amino acids in plasma and erythrocytes On fasting, the values for NE amino acids in erythrocytes rise to more than five times the levels observed on stock diet while the E amino acids increase two to three times In plasma the NE rise to three times but the concentration in the E hardly rises at all On the non protein diet, NE amino acids fall in the erythrocytes to about *one half* while the essential amino acids fall to less than one-sixth of the values shown by the animals on stock diet In plasma there is a much smaller fall, both in NE and E amino acids

DISCUSSION

The NE/E amino acid ratio has been proposed as a method for assessing the nutritional status in the preschool child and this index has been reported by various authors to be high in protein-calorie malnutrition Our results (Table 1) indicate that this ratio is seen (72 hours) altered by fasting and by protein deprivation in the rat both in plasma and erythrocytes However the increase in the ratios is brought about in very different ways by the two dietary treatments In fasting higher ratios are achieved by an increase in the concentration of NE amino acids with little or no decrease in the E amino acids On the other hand, in protein deprivation high ratios occur through a very marked decrease (Table 2) in the E amino acids, especially valine This type of change is very much like the one reported in PCM (6–8)

An increase in the erythrocyte/plasma distribution ratio in PCM has been reported by Bjornesjo (4) and recently also found in malnourished Iranian children (7) In our experiment, both fasting and protein deprivation produced marked changes in the erythrocyte/plasma distribution as can be seen from Table 3 but while fasting caused a decrease, the non protein diet induced a marked increase in the ratio especially for the essential amino acids The type of change reported is similar

Table 2 *Effect of fasting and a non protein diet on the concentration of amino acids (μg/ml) in plasma and erythrocytes (mean ± S E M)*

Group	*n*	Erythrocytes Gly	Ala	Val	Leu	Plasma Gly	Ala	Val	Leu
Fasting (F)	8	173±17	76±6 1	26±5 5	45±6 3	145±12	47±5 7	25±2 3	21±2 6
Non protein (NP)	8	17±2 4	7 0±1 4	2 0±0 3	2 0±0 37	48±0 74	17±2 3	7 0±0 37	12±0 96
Stock (S)	10	33±2 1	13±0 70	13±1 2	16±0 35	60±5 6	19±1 2	20±1 8	20±1 5
S vs F	*t*	8 3	8 7	2 4	5 5	7 0	5 0	3 5	0 4
	p	<0 001	<0 001	<0 05	<0 001	<0 001	<0 001	<0 01	N S
S vs NP	*t*	13 8	5 9	9 6	4 0	2 2	1 0	7 3	7 0
	p	<0 001	<0 001	<0 001	<0 001	<0 05	N S	<0 001	<0 001
F vs NP	*t*	7 1	11 6	4 4	6 8	8 1	5 8	7 9	3 6
	p	<0 001	<0 001	<0 001	<0 001	<0 001	<0 001	<0 001	<0 01

Table 3 *Amino acid distribution between plasma and erythrocytes (mean ± S E M)*

Group	n	Gly	Ala	Val	Leu
Fasting (F)	8	0 87±0 08	0 61±0 06	1 6±0 55	0 72±0 31
Non protein (NP)	8	3 2±0 32	2 4±0 09	3 6±0 48	5 8±0 89
Stock (S)	10	1 9±0 06	1 5±0 04	1 5±0 18	1.3±0 11
S vs F	*t*	25 8	22 0	0 2	2.0
	p	<0 001	<0 001	N.S	N.S
S vs NP	*t*	4 2	11 0	4 8	5 0
	p	<0 001	<0 001	<0 001	<0 001
F vs NP	*t*	7 3	26 0	7 4	6 0
	p	<0 001	<0 001	<0 001	<0 001

to the one found by Bjornesjo working with malnourished Ethiopian children (9)

Concluding the NE/E amino acid ratio (both in red cells and in plasma) and the amino acid distribution ratio are affected both by calorie and protein deprivation The changes that have been described in PCM in preschool children resemble more those produced in the rat by a non protein diet than by fasting Our results also seem to indicate that the reliability of the NE/E amino acid ratio both in plasma and erythrocytes as an index of PCM would be greatly influenced by the diet prior to testing Because the changes induced in our rats took only 3 days to become apparent it seems legitimate to assume that the ratios reflect more the quality of the diet than the nutritional status of the animals *per se* Similarly in children abnormal NE/E ratios (or plasma/erythrocyte distributions) would reflect more an inadequacy of the diet than an inadequate nutritional status

SUMMARY

The concentration and ratio of non-essential to essential amino acids were determined by paper chromatography in serum and erythrocytes of fasting rats and in animals that received a non protein diet and compared with rats on a stock diet

Our re[illegible]ow that very different changes are [illegible] in the non essential to essential amino acid ratios in the amino acid concentrations and in their relative erythrocyte/plasma distribution by the different dietary treatments The changes induced by the non protein diet resemble those reported in children suffering from protein-calorie malnutrition The fact that the changes are already apparent after only 3 days of dietary treatment seem to indicate that abnormal ratios reflect more the quality of the diet than the nutritional status

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INHERITANCE OF CHRONIC GRANULOMATOUS DISEASE IN FEMALES

Report of a Female Patient and the Leucocyte Function Studies in the Family

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The clinical and pathological syndrome of chronic granulomatous disease (CGD) has been reported in about one hundred patients of whom the large majority have been boys (7 16 18) This disease can be ascribed to a congenital defect in the bactericidal capacity of neutrophil granulocytes associated with a defect in the oxydative metabolism of these cells (12 13) An identical clinical and pathological syndrome has been described in a limited number of female patients with a neutrophil defect indistinguishable from that of males (3 5 10 20) The pattern of genetic transmission seems however to distinguish the male from the female form CGD being inherited as an X linked trait in males but as an autosomal recessive trait in females (3 5 24)

Reports of female CGD are too few to firmly establish the genetics and other modes of inheritance have been suggested for both male and female CGD (*vide infra*) Furthermore it has been questioned whether CGD takes a milder course in females We therefore report the clinical picture of an additional female CGD patient with leucocyte function studies carried out in the patient and her near relatives and compared with similar studies in six male CGD families

Holder of a research grant from the Michaelsen Foundation Copenhagen Denmark

CASE REPORT

L J born Sept 21 1966 to unrelated healthy parents as the third of three girls of whom the first is healthy whereas the second had multiple congenital malformations including omphalocele anal atresia bladder-exstrophy malformed vertebrae and pes equinovarus The family history is unsuggestive of enhanced susceptibility to infections Though she had recurrent hordeola the patient was regarded healthy till Sept 1969 when recurrent fever weight loss and persistent cough developed She was hospitalized Dec 1969 with pneumonia and a possible pulmonary abscess She did not improve upon antibiotic treatment and was referred to the Department of Paediatrics Arhus Kommunehospital

Over the next 5 months she had changing pulmonary infiltrations pleural effusion ostitis in one os hamatum left sided coxitis and panaritia Peripheral lymph nodes and tonsils were enlarged and the liver was slightly enlarged Macroscopy of pus from the ostitis revealed no micro-organisms and cultures from the pus and from the pleural effusion grew no bacteria including mycobacteria mycoplasmata or fungi No improvement followed treatment with lincomycin or methicillin plus fucidic acid but during a 65 day course of streptomycin treatment a slow regression of the pulmonary and osseous lesions occurred and she was discharged to her home

During the next year she had repeated skin infections panaritia and conjunctivitis and was readmitted May 1971 with infectious dermatitis especially pro-

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Fig 3 Tuberculoid granulomas in inguinal lymph node × 250

Fig 4 Pigmented lipid histiocytes × 400

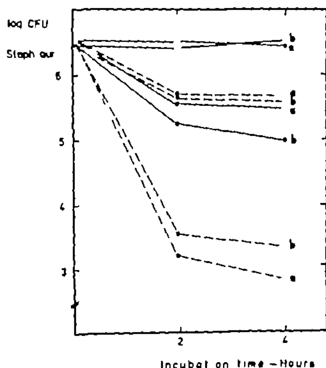


Fig 1 Results of *in vitro* leucocyte function studies in the patient Staphylocidal activity of peripheral blood leucocytes from the patient (•—•) and a normal control (o—o) — indicates total number of surviving colony forming units (*CFU*) *Staph aur* indicates intracellularly surviving CFU Two tests *a* and *b* were done with a 10-day interval

nounced around body openings an abscess on the thigh and peripheral lymph node enlargement Cultures from the abscess were negative but *Staphylococcus aureus* could be recovered from the nose and conjunctival sac Following local treatment of skin lesions she was discharged and there has been no progression of the disease till the present time

Laboratory investigations

On both admissions the erythrocyte sedimentation rate was increased Total leucocytes and absolute granulocyte counts were normal or slightly increased There was slight anemia Total serum gammaglobulin was 1 9 and 1 46 g/100 ml with slightly elevated IgG and IgA on first admission Also on first admission the antistreptolysin titre was increased (500) but fell to normal (125) and cold hemagglutinin titer was increased (128) The Moro tuberculin test was negative

Pathology

A biopsy from the groin contained a slightly enlarged lymph node with a normal fundamental structure and enlarged germinal centres The most remarkable feature was several more and less confluent granulomas of the tuberculoid type some with central necrosis (Fig 3) Scattered throughout the tissue sometimes adjacent to the granulomas were found pigmented histiocytes (Fig 4) and a few foam cells The number of plasma cells was increased and these were bigger than usual and some contained coarse granules of the Russel Fuch type The combination of tuberculoid granulomas and pigmented lipid histiocytes with the following staining reactions is diagnostic for chronic granulomatous disease (15 21)

Histological stainings

The results of the staining reactions applied are given in Table 1

Leucocyte function studies

The *in vitro* leucocyte function tests were done by a modification of the method of Alexander et al (1) as reported earlier (11) Dextran sedimented leucocytes from heparinized blood were washed twice in Hanks balanced salt solution containing 0 1 gelatin and 15 mg/100 ml heparin re suspended and adjusted to a concentration of 5×10^6 neutrophil granulocytes per ml *Staphylococcus aureus* strain 502 A or phage type 42 E+ fully sensitive to penicillin and streptomycin were grown overnight washed thrice in 0 9% saline and diluted in the above mentioned medium to give a final concentration of 3 to 6×10^6 colony forming units (CFU) per ml

Incubation mixtures consisted of 1 0 ml leucocyte suspension 0 8 ml bacterial suspension and 0 2 ml pooled normal human serum Duplicate incubation mixtures from the person tested and a normal control were set up in disposable plastic tubes at 35 C with end over end rotation at 20 rpm To one set of tubes penicillin 100 units/ml and streptomycin 100 µg/ml

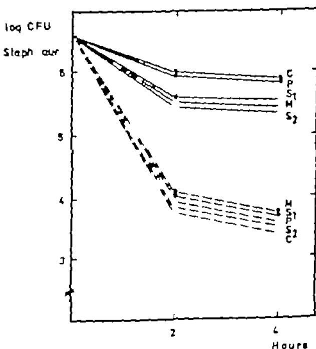


Fig 2 Results of *in vitro* leucocyte function studies in the family of the patient Staphylocidal activity of peripheral blood leucocytes from the father (*F*) the mother (*M*) two sisters (S_1 and S_2) and a normal control (*C*) — and as in text for Fig 1

DISCUSSION

With the typical clinical picture the granuloma formation and pigmented lipid histiocytes, and the decreased leucocyte bactericidal capacity this girl fulfils the diagnostic criteria of CGD It has been discussed whether CGD takes a milder course in females than in males In the present case the age of onset seems to have been somewhat delayed compared with most male patients if one disregards the early occurrence of hordeola (16) Furthermore there seem to have been rather prolonged periods of freedom from severe infections However the general clinical course has been quite characteristic with disseminated infection including osteomyelitis The protracted course and the location of lesions are typical as is the recovery of *Staphylococcus aureus* as a causative agent

Current methods of detecting the defective leucocyte function in CGD are 1) *in vitro* bactericidal tests 2) direct measurement of metabolic activity and 3) the histochemical nitroblue tetrazolium (NBT) test Studies by these methods have demonstrated a heterozygote carrier state in the mothers maternal grandmothers and some sisters of the large majority of male CGD patients Contrary to this a heterozygote carrier state could not be detected in the families of the first seven female CGD patients (3 5 10 20) Familial incidence and consanguinity suggested autosomal recessive inheritance (3 5) but dominance with variable expression (5) or spontaneous gene mutation could not be excluded Decreased NBT reduction was demonstrated in the mother and one brother of a recently reported female CGD patient and dominant inheritance with variable expression was again suggested (8) These family members were however not tested by leucocyte bactericidal tests

One group of investigators have reported abnormal neutrophil function in the fathers as well as mothers of seven male and one female CGD patients and have suggested a unified autosomal recessive inheritance for male and female CGD with sex modification leading to lethality *in utero* for female homozygotes (9 22) The method of demonstrating defective intracellular killing rate applied by these authors is however different from the more conventional techniques and this may account for these unexpected results (24)

Fig 1 and Table 2 clearly indicate the sensitivity by which the leucocyte defect is demonstrated by the method applied in these studies In six suspected carriers the lowest obtained ratio was 45 0 In marked contrast to this are the ratios of the mother and sisters of this female patient These results and the fact that two sisters do not show clinical or laboratory signs of a similar defect are compatible with autosomal recessive inheritance but also with spontaneous gene mutation The data would however not seem to fit with dominance with variable expression (3 8) and neither with autosomal recessive inheritance with sex modification (9 22) The patient could presumably be heterozygote for the X linked trait with uneven randomisation or Lyonisation resulting in a large proportion of cells carrying the defective gene (23) Speaking against this possibility is the fact that such a state has yet to be reported among suspected carriers in families of male CGD patients

Recent studies indicate that a difference may exist between male and female CGD on a metabolic level as well as a genetic Thus reduced activity of leucocyte glutathione peroxidase has been found in 2 female patients whereas normal activity was found in male patients (14) Precise evaluation of these findings should become possible when the underlying biochemical defect in male CGD becomes established (4 5 6 13) In view of the complicated series of events normally leading to the killing of ingested microorganisms it would however not seem surprising if different enzymatic defects might lead to the same functional and clinical manifestations and that different defects would be

Table 1 *Results of histological stainings of a biopsied lymph node from the patient*

HISTOLOGICAL STAININGS		
Routine stainings Hematoxylin-eosin Sirius red		
Staining reactions		
(A) *Pigment*		
Paraffin sections	Iron	−
	Fontana (Melanin)	−
Frozen sections	Sudan III	+
(B) *Histiocytes*		
Paraffin sections	PAS	+
	Toluidine orthochromatic	
	Alcian blue	−
	Ziehl Neelsen	(+)
	Autofluorescence yellow	
	Thioflavine	−
Frozen sections	Sudan III	+

Table 2 *Results of in vitro leucocyte function studies in the patient six male C G D patients, and some family members*

		Patient	Mother	Father	Sister 1	Sister 2
L J	Female	479 5[a]	2 5	1 3	1 7	1 3
M S	Male	177 7	159 6[a]			
M P J	Male	60 2[b]	69 5			
T S[c]	Male	929 0	63 3	2 2	45 0	60 0
B P[d]	Male	50 4[b]				
B V J	Male	198 5	56 5	0 8		
J R	Male	548 2[a]	314 7			

[a] Mean of two determinations
[b] Mean of three determinations
[c] This patient reported elsewhere (2)
[d] This patient reported elsewhere (11)

were added after 15 minutes incubation Samples were taken at 2 and 4 hours incubation time Total number of surviving CFU was determined from tubes without antibiotics by pour plating of appropriately diluted samples after lysis of the cells in sterile water Intra cellular surviving CFU were estimated in the same way from tubes with antibiotics after washing of the cells to get rid of the antibiotics

The nitroblue tetrazolium (NBT) tests were done according to Park et al (19) with modifications de scribed previously (17) Stimulated tests were done using bacteria free filtrates of bacterial cultures as previously described (17) and with heat killed *Candida albicans* and latex particles

RESULTS

Results of the leucocyte function tests on the propositus and the normal control are shown in Fig 1 Two tests were run with an inter val of 10 days There was no significant reduc tion in total surviving CFU within the ob servation time of 4 hours compared with con trol leucocytes which killed 88 % and 97 % of original CFU A large proportion of viable CFU (17% and 12% of starting values) could be recovered intracellularly from the patients leucocytes compared with control values of 0 02% and 0 08% Results of leucocyte func tion tests on the parents and the two sisters are shown in Fig 2 There is a normal reduc tion in total surviving CFU by all family members and recoverable surviving intracel lular CFU ranges from 0 16% in the mother to 0 064% in the control

The defective intracellular bactericidal ca pacity in CGD becomes evident when ex pressed as the ratio of surviving intracellular CFU in patients, over that in controls In Table 2 are given the results of leucocyte function tests done in 6 male CGD patients and some of their family members together with the results of the present studies in this family

The results are expressed as ratios of intra cellularly surviving CFU in each individual over a normal control The very high ratios are evident both in the affected males and this female ranging from 50 4 to 929 0 and in suspected female carriers ranging from 45 0 to 314 7 In marked contrast to these results are the results of the leucocyte func tion test in the mother of this female patient who showed a ratio of 2 5 The ratios for the father and the two sisters are comparable to the ratios of the two fathers of male CGD patients tested

The patient s neutrophils did not stain *in vitro* with NBT and stimulation of the cells with a battery of bacteria free filtrates from 12 different strains (17) or heat killed yeast and latex particles did not induce staining in more than 1 % of the neutrophils although ample uptake of the particles could readily be demonstrated

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linked to different genes giving rise to the observed genetic heterogeneity of this disease

It seems likely at present that two modes of inheritance are operative in CGD the most common beeing that of an X-linked, operative in the large majority of male patients, and an autosomal recessive beeing operative in most, if not all female, and a few male patients The present findings are in agreement with this

SUMMARY

A case of chronic granulomatous disease in a female child is presented Onset of symptoms seem to have been somewhat delayed compared with most male patients The results of *in vitro* leucocyte function studies on the patient and her parents and siblings are presented together with the results of similar studies on 6 male patients and some of their relatives In contrast to the male patients no heterozygote carrier state could be detected among the family members These results are in agreement with current views on the genetics of chronic granulomatous disease that the large majority of male cases are inherited as an X linked trait whereas most if not all female cases are inherited as an autosomal recessive trait Spontaneous gene mutation cannot, however be excluded in the present case

ACKNOWLEDGEMENTS

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Key words: Chronic granulomatous disease, females, genetic pattern

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LETTER TO THE EDITOR

We read with great interest the case report by Kaloustian & Mnaymneh (1) To date the reported cases have usually been familial (1–3) We report a presumably sporadic case

Case report M K was a four year old male born to consanguineous parents The mother was 30 years of age and the father 37 The pregnancy was uneventful she was not exposed to X ray and had not taken any drugs She also denied any infections during the earlier part of the pregnancy There were no other individuals in the family with similar malformations

Physical examination revealed a bilateral lobster claw deformity of the hands with absence of the middle fingers There was increased external rotation of both hips while the knees had a 90 degrees flexion contracture The feet were found to have marked equino varus deformity and two rudimentary toes

Radiograms of the lower extremities showed in addition to a marked muscular atrophy bilateral agenesis of the tibiae (Fig 1) The single bones in each crural region were believed to represent fibulae since they apparently formed no articulation with the femora proximally There was bilateral absence of the talus as well as agenesis of some metatarsal bones and phalanges Radiograms of both hands showed the presence of only four phalanges bilaterally There was a midline separation of these four digits producing a claw like deformity (Fig 2) A fairly large carpal bone present bilaterally probably represented fusion of two ossicles Other studies including EEG IVP intelligence test and chromosome analysis using peripheral blood revealed no abnormalities

The physicial and laboratory examination of the parents and the two sisters also showed normal findings

Sevgi Yetkin
Tugrul Pirnar
Burhan Say

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Fig 1 X ray of the lower extremities showing bilateral agenesis of the tibiae



Fig 2 X ray of both hands

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LETTER TO THE EDITOR

Sir

In the March issue of Acta Paediatrica Scandinavica Ekelund et al (3) reported the results of their investigation of fibrinolytic (plasminogen activator) activity in lung tissue from neonates with hyaline membrane disease The authors conclude that their findings do not support the role postulated for a lack of pulmonary plasminogen activator in the pathogenesis of hyaline membrane disease as I had originally proposed These conclusions were drawn by comparing the level of pulmonary plasminogen activator activity in the lungs of babies dying of hyaline membrane disease to that in a group of babies dying with pulmonary atelectasis but without hyaline membranes

It is of interest that 5 of 15 lungs with hyaline membranes showed no fibrinolytic activity whatsoever and 9 others had extremely low activity Ekelund graded fibrinolysis with a fibrin slide technique on a scale of I to III and assigned arbitrary units (A U) of fibrinolytic activity to each lung specimen by summing up the grades of fibrinolysis resulting from 4 individual lung sections incubated for increasing periods of time from each lung specimen Fibrinolytic activity of 4 A U or less would indicate that the observer probably found only Grade I or microscopical punctate areas of lysis in each of the sections of the lung at most Only 1 of the 15 hyaline membrane lungs had plasminogen activator activity in amounts comparable to that found in two normal lungs and the remainder caused only microscopic fibrinolysis The authors chose to ignore the normal lung group studying only two such normal lungs and base their conclusions upon a comparison with a series of 11 lungs showing atelectasis without hyaline membranes However only 3 of 11 lungs from the latter group had normal fibrinolytic activity one had no activity and the remainder caused only microscopic lysis

I question whether a comparison of hyaline membrane lungs to lungs with atelectasis is a valid comparison It is a well stated opinion that infants dying with atelectasis alone may be the result of an identical pathogenesis as those having typical hyaline membrane disease (1 2 5 6) Thus the fact that plasminogen activator was missing in 1 of 11 cases with primary atelectasis and was low in 7 others of this group certainly should not be used as evidence against the role of a deficiency of plasminogen activator in the pathogenesis of hyaline membrane disease I would suggest that Ekelund and co workers expand their normal control group with lungs from newborn fetuses and infants succumbing to non pulmonary pathology if an adequate comparison is to be made

The conclusion that I would draw from Ekelund s study is that *a deficiency of pulmonary plasminogen activator activity occurs frequently in infants with hyaline membrane disease and possibly in infants dying from primary pulmonary atelectasis* No single factor can account for a specific type of pathology in all instances so that this deficiency need not be present in every case to be significant a deficiency of surfactant is not found in every hyaline membrane lung either Further confirmation of my previous findings could be obtained if the authors would mix homogenates of the inactive lungs from hyaline

membrane babies with normal lung homogenates to demonstrate the presence of an inhibitor to the plasminogen activator I have found such an inhibitor in the majority of hyaline membrane lungs lacking plasminogen activator activity (4)

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The Editorial Board has asked Dr Ekelund to comment on Dr Lieberman's Letter to the Editor

Sir

We do not agree with Dr Lieberman that the fibrinolytic activity was extremely low in 9 cases of hyaline membrane disease (HMD) In fact 6 of these cases had activities ranging between 3 and 4½ AU (Fig 1) i e clear fibrinolysis thus disproving total lack of plasminogen activator activity as well as the presence of large amounts of fibrinolytic inhibitors Considering also the case of HMD with high fibrinolytic activity (9 AU) the association between HMD and low fibrinolytic activity is not yet completely clear

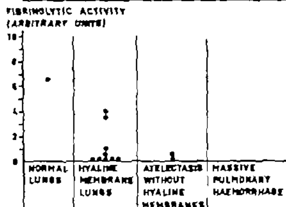


Fig 1

We agree with Dr Lieberman that infants dying with atelectasis alone may be the result of an identical pathogenesis as those having typical HMD which is also pointed out in our article (p 155) Dr Lieberman states (1) that a deficiency of plasminogen activator activity could play a role in the retention of intrapulmonary fibrin deposits and the formation of hyaline membranes Assuming this deficiency to be a common pathogenetic factor in atelectasis and HMD one might wonder why lungs with atelectasis alone do not show hyaline membranes

We are well aware of the smallness of the normal material but in our country it is very rare for neonates to die from causes other than lung disorders Even during a much longer period Dr Lieberman himself (1) has been able to collect only 7 normal control lungs His pathological material consists of 11 lungs compared with our 27

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LETTER TO THE EDITOR

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Fig 1 Clinical course GVH designates the period of graft versus-host reaction

... continued correction of the fluid supply Even then the child had episodes of preshock following ex... ...ous diarrhoea

In order to have an approximate value for fluid loss from rectum and bladder (called output) the diapers were weighed before and after use Even if exact distinguishing between urine and stool volume was not always possible it was evident that polyuria was only present in connection with a colistin intoxication Output thus represents diarrhoea plus a near normal urinary volume

Output values are presented in Fig 2 After decontamination a gradual decrease was seen followed 10 days later by an increase surpassing 2 000 ml on day 59 (body weight 3 750 g) After discontinuation of non absorbable antibiotics prior to recontamination (day 60) there was a decrease in diarrhoea until a colienteritis again caused enormous diarrhoea

Fig 2 Fluid supply and loss of fluid by diarrhoea and urine (output)

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CASE REPORT

PROLONGED DIARRHOEA IN A CHILD WITH SEVERE COMBINED IMMUNODEFICIENCY

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Diarrhoea is a frequent symptom in children with severe combined immunodeficiency (CID) We present here a case where the diarrhoea was excessive, requiring a daily fluid supply of about 60° of the bodyweight Due to intensive fluid therapy and isolation in germ free environment the child survived for 3 1/2 months, permitting an attempt of immunological reconstitution

CASE HISTORY

This girl (M K J) born 1970-10 21 was a first child of healthy parents Birth weight 3 800 g Except for persistent rhinorrhoea the first 2 months were uneventful but the child gained only 800 g At 3 months of age she developed oral thrush and her stools became loose At 4 months of age she was admitted to the University Clinic of Paediatrics Copenhagen County Hospital Gentofte with pneumonia She had lymphopenia negative response of lymphocytes to phytohemagglutinin (PHA) and to allogeneic lymphocytes with low serum IgG and absent IgA and IgM in serum thus establishing the diagnosis of CID (10)

The child was referred to the University Clinic for Infectious Diseases Copenhagen for germ free isolation and possibly immunological reconstitution

Upon admittance 5 /4 months old she was chronically ill with a weight of 3 800 g She had abscesses where intravenous catheters had been in place growing *E coli* and *Staph aureus* A variety of other micro-organisms including *Candida albicans* could be demonstrated

The child was placed in a laminar air flow bench Persons handling the child were dressed with caps masks sterile overgown and sterile gloves and her food and medicine were sterile She underwent extensive decontamination (Fig 1) according to bacteriological findings and all bacteria were apparently eradicated however the particular strain of E coli demonstrated on admittance reappeared whenever treatment with non absorbable antibiotics were stopped No exogenous micro organism could be demonstrated during the 3 1/2 months of isolation Mycostatin was given during the entire period

During the first 2 weeks the child had episodes of hypoglycaemia which later disappeared spontaneously

Because of the invariably fatal outcome of CID a bone marrow transplantation (BMT) was performed 14 days after admittance There were only signs of a minimal take of the graft and a second BMT took place 26 days later using the same donor After approximately 4 weeks the PHA response was weakly positive Simultaneously she developed signs of a graft versus host reaction (GVH) with a typical exanthema followed by oedema and scaling She had a short period of neutropenia and from day 38 after BMT 2 persistent thrombocytopenia

During the GVH antibiotics were discontinued and recontamination attempted however cultures from the blood spinal fluid and stools soon grew *E coli* Vigorous treatment with antibiotics was started (Fig 1) with only temporary effect and the child died 9 months old with *E coli* septicaemia

Autopsy confirmed the diagnosis of CID Changes consistent with a mild GVH were present in the skin ileum and liver In the ileum hyperplastic glands alternating in places with atrophic and irregularly dilated glands and infiltration of the stroma with eosinophilic granulocytes were seen (Dr M J de Vries the Netherlands) There were scattered abscesses in the lungs and signs of endocarditis *E coli* could be cultured from a variety of organs

Diarrhoea – Diuresis

During the entire isolation period the child had profuse watery diarrhoea in greatly varying amounts neces

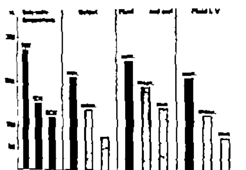


Fig 4 Mean values of body water compartments in the patient between day 30 and day 60 compared with fluid loss by urine and diarrhoea (= output)

loss with oedemas. Her length was unchanged at 62 centimetres

DISCUSSION

The enormous diarrhoeas were one of the big problems in this child and various etiological possibilities were considered

Such diarrhoea has previously been described in children with CID in connection with decontamination the sterilisation per se therefore afforded an explanation However we have since decontaminated another child without producing diarrhoea using the same non absorbable antimicrobials

Malabsorption may be produced by neomycin (9) and discontinuation resulted in a considerable decrease in the diarrhoea

The voluminous watery diarrhoea suggested secondary disaccharidase insufficiency due to infection and malnutrition (3) Furthermore disaccharidase insufficiency has been described in 2 children with CID (6) In mice with runt disease lactase insufficiency has occurred (1) During the 6 days when the child only had glucose orally no definite effect could be observed Prolonged trial with glucose alone or exclusive intravenous nutrition was not attempted in this malnourished child as partial absorption of protein fat and minerals do occur even when severe diarrhoea is present (4)

Exclusive intravenous feeding was never attempted, since the programs described by Wilmore & Dudrick (13) and by Filler et al (7) could not be carried out as the child did not tolerate hypertonic intravenous glucose Intralipid was avoided for fear of possible side effects

In intestinal GVH diarrhoea is always present but GVH cannot be the only explanation since severe diarrhoea was present before BMT

Because of the life threatening diarrhoea the necessity for intravenous catheter offering a port of entry for infection and the progressive risk of development of intractable diarrhoea recontamination was attempted since cessation of diarrhoea has been demonstrated in infants with CID when recontaminated with apathogenic bacteria (5)

After discontinuation of antibiotics prior to recontamination *E coli* reappeared in the stools and could soon be demonstrated in the blood and spinal fluid As previously pointed out by Solberg et al (12) the intestinal lesions during a GVH facilitate absorption of microorganisms thereby further increasing the susceptibility to infection

CONCLUSION AND SUMMARY

Prolonged diarrhoea in an infant with severe combined immunodeficiency is described necessitating a daily intravenous fluid supply of about one third of the body weight for 3 1/2 months It was possible to maintain a reasonable fluid and electrolyte homeostasis but the child died of an overwhelming infection before the diarrhoea could be successfully combated

The diarrhoea was probably caused by infection primarily continuing due to malnutrition Neomycin and a graft versus host reaction further aggravated the diarrhoea which probably finally became intractable as described by Avery et al (2)

The clinical course demonstrates that recontamination should never take place during a graft versus host reaction It also demonstrates that if possible intraveous catheters

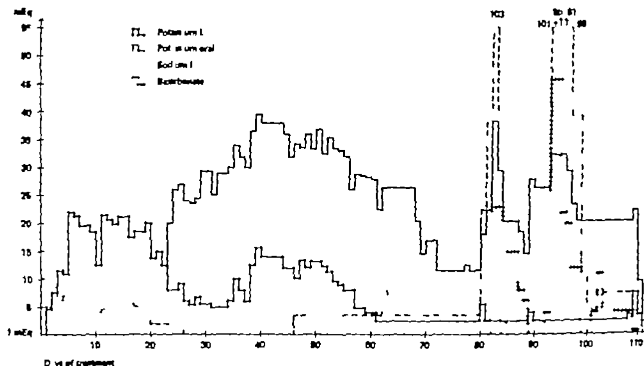


Fig 3 Daily supplements of potassium sodium and bicarbonate

On day 93 the child was severely dehydrated but in spite of this her diuresis was about 50 ml per hour The polyuria subsided after discontinuation of i v colistin Her maximum output was 2 760 ml per day

The diarrhoea increased in connection with a change in diet from human milk to Velactin® A decrease took place in a period of 6 days when she only had glucose orally but simultaneously neomycin was discontinued and the diarrhoea did not increase when human milk was started again

Fluid and electrolyte therapy

Through catheters in v cava the child had hypertonic glucose protein hydrolysate (Aminofusin®) and electrolytes (Fig 3) Local oedema developed but after reduction of the intravenous supply of potassium and discontinuation of hypertonic glucose the oedemas subsided and a catheter in the superior v cava functioned for more than 2 months when it had to be removed due to a local abscess For the following 5 weeks fluid supply was given through butterflies

The total and intravenous fluid supply is shown in Fig 2 The greatest amounts of fluid were given concomitant with the colienteritis and the colistin induced polyuria where approximately 3 300 ml were given per day the intravenous amount being approximately 2 800 ml

From day 30 to day 60 the weight was relatively constant (3 705 g) and the child had no oedema According to the data of Friis-Hansen (8) we estimated that the total body water (TBW) was approximately 2 700 g the extracellular water (ECW) being 1 200 and the intracellular water (ICW) being 1 500 g I this period the average total fluid supply per da was 1 856 ml of which 1 245 ml was given intr venously coorresponding to 155 and 104 of EC respectively and to 50 and 33 respectively of bod weight (Fig 4) In the same period the average ou put was 1 370 ml equivalent to 114 of ECW an 37 of bodyweight

Intravenous calcium supply was seldom necessar Magnesium supplement was 1 7 mEq orally and 0 mEq i v per day She never experienced convulsio nor twitching of the limbs

Due to the frequent changes in electrolyte suppl the measured serum electrolyte values were bei surprisingly constant

In spite of the enormous fluid supply the chil was never clinically overhydrated Oedemas causin weight gain around day 15 and day 70 were due t local traumas and an attempt to reduce fluid suppl resulted in preshock During her last days the chil was severely dehydrated but increased fluid suppl caused pulmonary oedema

Weight – Nutrition

The patient was fed human milk 660 ml per day Vitamins were supplied orally and parenterally Albu min was periodically given intravenously due to hypo proteinaemia Iron was given orally intramuscularl and she also received 9 irradiated blood transfusions

Upon admittance her weight was 3 800 g and durin the 3 / months she only gained weight in combina

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CASE REPORT

QTU ABNORMALITIES SINUS BRADYCARDIA AND ADAMS–STOKES ATTACKS DUE TO VENTRICULAR TACHYARRHYTHMIA

GÖTZ VON BERNUTH GUSTAV G BELZ WALTRAUD EVERTZ and MARTIN STAUCH

From the Department of Paediatrics and Section of Cardiology Zentrum fur Innere Medizin und Kinderheilkunde University of Ulm Ulm Germany

In 1957 Jervell & Lange Nielsen (6) first described the syndrome consisting of congenital deaf mutism prolongation of the QT interval in the electrocardiogram and syncopal attacks Later it was found that QT prolongation and tachyarrhythmic Adams–Stokes attacks may also occur in patients with normal hearing (12 13) This report concerns a young girl with tachyarrhythmic Adams–Stokes attacks normal hearing normal resting QT interval and a striking sinus bradycardia

CASE REPORT

A 10-year-old girl was referred to the University Hospital because of a cardiac arrhythmia detected during introduction of anaesthesia for tonsillectomy At the age of 1 1/2 years an unusually slow pulse was first noted Since the age of 3 years she had frequent attacks ranging in severity from brief dizziness pallor and nausea to sudden episodes of unconsciousness These attacks always occurred in connection with physical exercise or excitement The child was believed to have epileptic seizures and was treated without success with various anticonvulsive medications even though the electroencephalogram was normal

There is no history of deafness syncopal episodes or sudden death in the family on either side The patient s father has a sinus bradycardia of approximately 50 beats/min. He and his mother as well as the patient s two brothers show a slightly prolonged QT interval (QTc 0 43–0 45 sec)

The child s physical examination was unremarkable except for a slow heart rate of 50 beats/min Electroencephalogram and routine laboratory studies were normal except for a microhaematuria for which no cause could be found On the chest roentgenogram the heart was of normal size and configuration The electrocardiogram at rest revealed sinus bradycardia of 48 beats/min (Fig 1) The PQ interval was short (0 12 sec) but still within the normal range The T waves were rather flat but there were prominent U waves as is often seen with bradycardia The QT interval was not prolonged (QT 0 45 sec QTc 0 40 sec QT ratio 1 00) During exercise on a bicycle ergometer with a load of 50 watts the regular sinus rate rose to 90 to 100 beats/min while the QTc increased up to 0 53 sec and the QT ratio up to 1 32 The U waves remained prominent At a heart rate of 90 to 100 beats/min ventricular arrhythmias regularly developed either as uni or multifocal bigeminy (Fig 2 *a*) or as short runs of multifocal ventricular beats (Fig 2 *b*) or on one occasion as multifocal (Fig 3 *a*) and partly bidirectional (Fig 3*b*) ventricular tachycardia of approximately 200 beats/min

SPECIAL STUDIES

Right heart catheterization revealed no evidence of organic heart disease Electrical stimulation of the superior vena cava–right atrial junction resulted in an atrial rhythm with 1 1 conduction to the ventricles up to a frequency of 170 beats/min when Wenckebach periods appeared At no time were extra systoles observed Up to a frequency of 130 beats/min the T waves could be separated from the P waves at this frequency QT was 0 32 sec QTc 0 47 sec and the QT ratio 1 18 Intravenous injection of Atropine (twice 0 5 mg) increased the regular sinus rate to 108 beats/min (QT 0 36 sec QTc 0 48 sec QT ratio 1 20) without appearance of extra-systoles Intravenous infusion of a small amount of a beta adrenergic stimulator (Orciprenalin = isomer of iso-

should be avoided at least until decontamination has taken place

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I II III aVR aVL aVF V1 V2 V3 V4 V5 V6 1sec

Fig 1 Standard electrocardiogram at rest

proterenol 0 10 mg) raised the sinus rate to 91 beats min (QT 0 38 sec QTc 0 37 sec QT ratio 1 18) and within 2 min produced a multifocal ventricular tachycardia of approximately 190 beats/min which lasted 2 min and was preceded and followed by ventricular extrasystoles occuring partly as bigemini On a later occasion intravenous injection of 0 075 mg Orciprenalin resulted in mostly unifocal ventricular bigemini

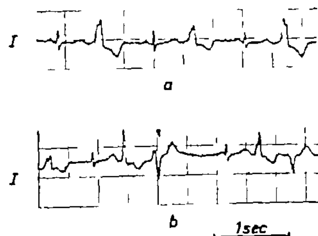


Fig 2 (*a*) Ventricular bigemini after exercise (*b*) Multifocal ventricular extra systoles after exercise

These ventricular extra systoles could not be s pressed by atrial pacing However when the sa dose of Orciprenalin was injected after prior int venous administration of a beta receptor block agent (Practolol 10 mg or Alprenolol 3 mg) no ex systoles were observed despite increase of the si rate to 120 beats/min

THERAPEUTIC TRIALS

The reproducibility of the patients ventricular ex systoles with exercise gave the opportunity of a quantitative evaluation of the therapeutic effect various drugs After an adequate period of tre with the respective drug the child was exercised 50 watts on a bicycle ergometer exercise was continued when the first extrasystoles appeared time interval from commencement of exercise to pearance of extrasystoles and from end of exer to disappearance of extrasystoles was measured T 1 gives a summary of the results It is evident when one drug at a time was used only the b receptor blocking agents Practolol Pindolol and l pranolol had a definitely positive effect on the rhythmia as indicated by the rapid disappeara of the ectopic activity after end of exercise No d of the QT produced any significant alteration of the val during rest or exercise compared to the

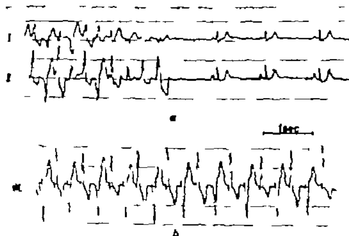


Fig 3 (a) End of multifocal ventricular tachycardia after exercise Note that immediately after restoration of sinus rhythm the QT interval is remarkably short (heart rate 60 beats min QT 0 34 sec QTc 0 34 sec QT ratio 0 85) (b) Bi-directional ventricular tachycardia after exercise

[illegible] state The combination of a beta receptor blocking agent (Practolol) with Verapamil showed the best clinical results With this regimen the child has now been normally active without any attacks for 13 months

DISCUSSION

Our patient's clinical history and to some extent her findings resemble those of patients with syncopal attacks and prolonged QT interval However the case is remarkable in two respects firstly there is no prolongation of the QT interval at rest and secondly there is a striking sinus bradycardia at rest

The QT prolongation although considered an essential part of the syndrome is of variable degree among different patients and often within the same patient. It may actually be rather slight (2) Some authors also mention the possibility that the impression of QT prolongation might be created by merging of

Table 1 *Time interval form commencement of exercise to appearance of extra systoles and from end of exercise to disappearance of extra systoles under various medications*

Only data for the highest dosage of the respective drug used are recorded The work load was always 50 watts

Drug	Dose (oral) mg/day/	Heart rate at rest (beats/min)	Heart rate before onset of systoles (beats/min)	Onset of extra syst after start of exercise sec	Type of extra syst	Disappearance of extra systoles after end of of exercises sec
No drug	–	50	105	37	ES	6
Verapamil	3 80	4	106	34	Big	56
Alprenolol Ajmalin bitartrat	0	67	107	6	ES	-4
Procainamid	4 500	90	104	32	ES	34
Procainamid and Digoxin	4 250 0	46	105	3	ES	46
Beta receptor blocker						
Practolol	4 100	49	95	48	Big	4
Pindolol	5 5	50	93	31	Big	2
Propranolol	4 40	48	90	40	Big	7
Practolol and Verapamil	3 100 3 80 40	47	95		—	—

ES [illegible] of multifocal ventricular extra systoles
[illegible] unifocal or multifocal ventricular bigeminy
[illegible] after 9 sec before exercise was discontinued

Fig 1 Standard electrocardiogram at rest

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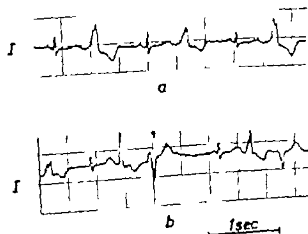


Fig 2 (a) Ventricular bigemini after exercise (b) Multifocal ventricular extra systoles after exercise

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an unusually prominent U wave with the T wave (6 5 9) In our case the U wave was well separated from the T wave and some times appeared abnormally prominent at rest The QT interval at rest was found to be with in normal limits for the heart rate With ex ercise atrial pacing Orciprenalin and Atro pine, the QT interval invariably shortened while the QTc rose to levels of up to 0 53 sec This QTc is above the upper limit of normal after one minute of exercise (16) The U waves during exercise usually remained pro minent or became more so

Our patients second striking feature is her sinus bradycardia at rest A slow resting sinus rhythm has been noted repeatedly in patients with QT prolongation both in the presence (10 11 7 8 1 15) and in the absence (13 8) of deafness as well as in one child with tachyarrhythmic syncopes without QT pro longation (14) An association of sinus brady cardia at rest and a tendency to ventricular tachyarrhythmias under physical or emotional stress thus appears to be more than coinci dental The mechanism of this association re mains unclear but it is tempting to assume sinus node disease especially since James (4 11) found minute infarctions in and around the sinus node in such cases

While increase in heart rate other than by sympathetic stimuli (atrial pacing and Atro pine) did not trigger ventricular extra systoles in our patient physical exercise and small doses of a beta adrenergic stimulator regu larly did Thus, an undue sensitivity of the myocardium to sympathetic stimulation, as proposed by Ward (13) may be postulated The beneficial effect of beta receptor block ing agents supports this hypothesis Such un due sensitivity may be related to the repolari zation abnormality suggested by an abnor mally prominent U wave and a prolonged QT interval during exercise Stimulation of adren ergic nerves increases the degree of temporal dispersion of repolarization (3) Intravenous catecholamines produce the same effect with in 2 minutes of the start of infusion although the dispersion is significantly reduced then after (3) It is believed that an increase i temporal dispersion of repolarization predi poses to ventricular ectopic beats The like liest mechanism for this effect is re-excitatio of already repolarized elements by the flo of currents between these and neighborin elements which are still depolarized (4)

It is postulated that in this as in other pa tients with QTU abnormality a pre existin non uniformity of repolarization is aggravate by sympathetic stimuli resulting in ventricula tachyarrhythmia

SUMMARY

Tachyarrhythmic Adams-Stokes attacks, pro voked by physical and emotional stress are reported in a 10 year old girl with sinus brady cardia at rest, prominent U waves, a normal resting QT interval but QT prolongation on exercise Two brothers the father and the paternal grandmother have a slight QT pro longation but no attacks In the proband physical exercise and small doses of a beta adrenergic stimulator reliably cause ventric ular bigemini and/or runs of multifocal ven tricular extra systoles while increase in heart rate by Atropine and atrial pacing does not The ventricular arrhythmia is improved by beta receptor blocking agents It is proposed that in this and other patients with abnormal repolarization the ventricular myocardium is unduely sensitive to sympathetic stimuli, re sulting in ventricular tachyarrhythmia

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√C A Smith (ed) *The critically ill child* and management 276 pp illus W B Sa Company Philadelphia London Toronto £5 00

√M O Tachdjian *Pediatric orthopedics* vol 1 766 illus vol 2 1001 pp illus W B Saunders C pany Ltd London 1972 £27 65

Bo Vahlquist (ed) *Nutrition A priority in Afr development* 227 pp illus Almqvist & Wik Stockholm 1972 Price not given

Genetic disorders Prevention treatment and rel tation World Health Organization Technical port Series no 497 Geneva 1972 40 pp

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ANNOUNCEMENT

INTERNATIONAL PRIZE FOR MODERN NUTRITION

A prize of Sfr 15 000 will be awarded again in September 1974 by the Central Union of Swiss Milk Producers *Berne* to a scientist from one of the following countries members of the International Dairy Federation Argentina Australia Austria Belgium *Brazil Bulgaria* Canada Czechoslovakia Denmark Finland France India Ireland Israel Italy Japan Kenya Luxemburg Netherlands New Zealand Norway *Poland* South Africa Spain Sweden Switzerland United Kingdom USSR West Germany

The subject chosen for the 1974 prize is *Milk and lactation* All scientists (chemists physicians bi logists) who have worked in this field are eligib Applications should be sent to the President of jury Professor M Demole Unite de Dietetiq Hopital Cantonal CH 1211 Geneva 4 until Janu 31 1974 with 3 copies of *(a)* Curriculum vit *(b)* List of works *(c)* Reprints of 2 or 3 papers the theme of the prize published in the last 5 yea (no typewritten papers) These documents should b written in *English French* or *German* or should accompanied by a translation into one of these languages (They will not be returned to the authors